

A MONOGRAPHIC STUDY OF SPECIAL GROUPS OF THE WATER MOLDS

I. BLASTOCLADIACEAE

II. LEPTOMITACEAE AND PYTHIOMORPHACEAE

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A MONOGRAPHIC STUDY OF SPECIAL GROUPS OF THE WATER MOLDS I. BLASTOCLADIACEAE¹

BESSIE BERNICE KANOUSE

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INTRODUCTION

Among the water molds, which include the well-known members of the Saprolegniales, there are groups concerning which we have very incomplete information. The life-histories of a number of them have remained uncertain; their relationships, as a result, are poorly understood; and their geographical distribution in this country can only be surmised. Since the writer has been able to find and study nearly half of all of the known species, to extend very materially their geographical range, and to discover new members of the group, it seems advisable to reorganize all of our data of these water molds into a more or less monographic whole.

In an extensive study of any group of the aquatic fungi, it is essential that their development and reactions should be followed under controlled conditions. This is the more necessary because, in most cases when they are found growing out-of-doors on some chance substratum or when caught by artificial bait, more than one species, or even more than one genus, is present. Indeed, at times a considerable number of water molds may be found growing on a single object. Each individual form should obviously be isolated in pure culture in order not to draw facts from more than one species at once. In many cases, however, this isolation has not yet been satisfactorily made.

TECHNIQUE AND EXPERIMENTS

When the work of collecting these forms was first started, it was assumed that it would be only a relatively brief time until a method could be devised for obtaining them in pure culture, and that soon one could begin to work out in such favorable cultures the physiological and morphological details of their life histories in the same manner in which *Pythiomorpha gonapodioides* Petersen was studied (15). This hope gradually weakened, inasmuch as it became evident that the behavior of these fungi was of a radically different type

¹ Papers from the Department of Botany of the University of Michigan, no. 249.

from that of the species belonging to the Saprolegniaceae, Pythiaceae, and similar groups. For a detailed account of the field work and its accompanying technique the reader is referred to a previous paper (16).

In order not to lose the valuable opportunity of studying such large numbers of forms, at least to some extent, after the method of collecting them had become easy, it was determined to develop a method by which the plants brought indoors could be kept in excellent growing or living condition on the substratum on which they had been found, and thus make possible that thorough acquaintance with these fungi which is necessary before substantial contributions can be made to our knowledge of them or their species accurately determined.

It was found that these fungi can be kept satisfactorily in rough culture in the laboratory for a long time, and hence the bait-setting may be so distributed that at any time throughout the entire year it is possible to have adequate material on hand for study. Investigations concerning the morphology and taxonomy were based, therefore, not only upon examination of the collections as they were brought directly from the water, but upon repeated reexaminations of a large number of those collections at later times. This procedure extended over a period of three years so that the normal types as well as the variations of the twelve species collected were critically studied.

The following paragraphs present the method of treating the cultures in the laboratory and the use that was made of some of them in attempts to get them into pure culture.

The treatment of the material after it is brought into the laboratory determines to a great extent the usefulness of the collections for further study. Each collection was kept in the laboratory by first carefully washing the bait (apple, prune, or other material) in tap water and then placing it in a large, covered, glass capsule containing about 200 cc. of sterile distilled water. Later experience showed that the collections could be kept with greater safety if water from the place of collection was brought into the laboratory and used instead of the distilled water. Contaminations of *Penicillium* and other fungi are minimized if the bait is at all times kept covered completely with water and if the culture dishes are placed immediately in a cold room. The temperature in the cold room ranged from 2° to 8° C. Many of the cultures were kept successfully in a cold frame built inside a large window with a section opening to the outside by means of which the light and temperature could be partially regulated. Here the temperature during the winter months varied from 8° to 15° C. In both situations the cultures kept nicely if the water was changed frequently. Microscopic examinations of the collections should be begun immediately after they are brought into the laboratory, for the sudden changes brought about by the new indoor conditions usually result in rapid and important developments. The earlier they can be examined, too, the freer they will be from contaminations.

Detection of the colonies of these fungi on the fruits is not always as simple a matter as would appear from the above statement. The fruits were usually covered with dense mats of filamentous water molds or with thick layers of mud, always accompanied by protozoa, bacteria, and other organisms. The first observations would often fail to discover the presence of any fungus whatsoever. Under favorable circumstances, the presence of some of these forms is indicated by small wart-like roughenings on the skin of the fruits. Occasionally the bait is thickly dotted with large white patches of mycelium plainly visible to the eye. Identification of genera, of course, is always dependent upon a microscopical examination.

The cultures must be washed thoroughly and frequently while they are kept indoors, to remove the excess of contaminating and injurious organisms. However, there are certain iron bacteria that do not seem to be harmful, as a thick covering or scum can form on the surface of the water in the culture dishes without immediately killing the fungi. Apples were found to be the most favorable substratum, although rose and *Crataegus* fruits were also used successfully. The latter are less desirable as the fungi die sooner on this bait than on either of the other kinds just mentioned.

New infections occur in the laboratory cultures on the fruit surfaces and as a result fungi can be had for continuous study. It is certain that the new plants are not injured by the culture conditions described above as they appear in all respects like those obtained in outdoor collections, where we must assume they have grown under favorable conditions for development.

In such cultures it is obviously impossible to determine the causes of the presence or absence of reproductive organs. Sporangia were abundantly formed in nearly all of the fungi studied. It is not easy, however, to regulate the conditions affecting the escape of the zoöspores. It appears from the writer's experience that these fungi are extremely sensitive to external conditions. They are usually so disturbed by the removal from the fruit to a slide that the protoplasmic activity is arrested, and spores, even in advanced stages of development, fail to become discharged. In the case of *Blastocladia* spp. the spores swarm abundantly and readily in cultures that are two weeks old or more after the plants are transferred to distilled water and the temperature of the room in which the study is being pursued is considerably below normal. Petersen (27), however, states that zoöspores of *Blastocladia Pringsheimii* Reinsch seldom swarm.

Sexual organs are sometimes abundant in the collections brought directly from the lakes. Sometimes, however, they are formed in the laboratory cultures after being indoors for months, but frequently they are never observed. It is more than probable that certain environmental conditions control their formation, although until these fungi can be grown and studied in pure culture, it is impossible to determine what those conditions are.

From cultures kept in the manner above described, on the substrata on which they were originally found, were made those studies of which the results appear in the taxonomic portion of this paper.

There are special difficulties attending efforts to secure pure cultures of these fungi that are not experienced in the Saprolegniaceae. These difficulties are partly due to the fact that we still know but little concerning the nutritional requirements of this group. It is more than probable that certain physical phenomena play an important rôle in the technique required for successful cultures. The problem of getting pure cultures is especially difficult in the case of those species which develop extensive rhizoidal systems. The fact that small portions of such plants do not grow by rejuvenation of isolated parts, that sporangia do not function as conidia, that oogonia do not readily develop into plants, together with our limited understanding of the substances that are used by these fungi in the elaboration of food, indicate the type of difficulties to be met with in experimentation. However, the crucial point around which the important physiological reaction centers is the behavior of the protoplasm in the sporangium after the spores are nearly formed, as well as in the spores after they are liberated from the sporangia. In plants carefully mounted for microscopic examination, spores that are apparently in a late stage of development are prevented from being fully formed and seldom are released from the sporangia. Those that are released are fragmented easily when introduced into sterile solutions or when sprayed on agar in petri dishes, so that it is quite impossible to use spores as the starting point for experiments. Not only in the swimming stage but also in the stages after germination the protoplasm is still extremely sensitive and is so disturbed by handling that plants do not continue their development. Such organic responses are markedly different from those obtaining in similar attempts to secure single spore cultures in the Saprolegniaceae.

The genera *Leptomitus* and *Apodachlya* are represented by plants that have a filamentous vegetative structure and are more easily obtained in pure culture. *Leptomitus lacteus* has been studied in such cultures by Kolkwitz (21, 22, 23) while Coker (7) and the writer have obtained and studied similar cultures of *Apodachlya brachynema*.

Coker (7) describes briefly the reactions of *Sapromyces Reinschii* in culture and states that the plant grows slowly in vegetable juices obtained by boiling corn grains or peas.

We are indebted to von Minden (26) for a detailed account of the physiological experiments on the genus *Araiospora*. He secured vegetative growth and asexual reproduction in pure culture of *A. spinosa* grown either on solid or in liquid media. Von Minden also claims to have obtained pure cultures of *Rhipidium europaeum* by methods similar to those used for the culturing of *Araiospora* sp. Those methods were tried by the writer, together with extensive experiments involving modifications of his technique, but for some reason no pure cultures of either *R. europaeum* or *R. americanum* were obtained.

In the family Blastocladiaceae the writer attempted to get *Blastocladia*

Pringsheimii into pure culture by using a technique similar to that employed in the experiments with species of *Rhipidium* but with the same negative results. Members of the genus *Allomyces* have been successfully cultured by Barrett (1) and by Coker (6).

A comparison of the results of the cultural experiments on all the members of the Leptomitaceae makes it clear that they possess to some extent, at least, similar physiological characteristics. The exact nature of these common characteristics is as yet a matter of speculation. Even though the secret of cultural experimentation should be determined for any one fungus, there is no reason for supposing that the same technique in detail can be applied with success throughout the whole group, nor that the results, aside from the fundamental principles involved, will necessarily be alike. Klebs (19, 20) showed conclusively that the reactions of plants in culture are unquestionably specific and that the inferences drawn from the study of one species do not necessarily apply to others.

MORPHOLOGY

Through the investigations carried on by the writer, several contributions have been added to our knowledge of the morphology of the Leptomitaceae and Blastocladiaceae. These observations not only give a new meaning to the interpretation of the morphological facts of the genus *Blastocladia*, but radically change our conception of the family Blastocladiaceae. This paper establishes for the first time the occurrence of antheridia in that family. Since adequate evidence for the presence of the male gamete has never previously been reported, there has been only speculation regarding the nature of the oöspore-like bodies that are known to belong to all species of *Blastocladia* and *Allomyces*. These organs have been called "resting spores" by most authors, while others (5, p. 1030) have regarded them as oöspores which are parthenogenetically formed. In most cases it was intimated, if not plainly stated, that in all probability sexuality is degenerate in this family and is no longer expressed.

Some weight attaches to this argument because of the fact that the oöspore-like bodies are different morphologically from the true oöspores known in certain genera of the Leptomitaceae. The distinguishing morphological character of these oöspore-like structures, in certain species of the Blastocladiaceae, is the slipping out of the oöspore from the oögonial wall before germination. Such a behavior is unique and is known in only one other family of fungi, *viz.*, the Monoblepharidaceae. It has been reported for *Blastocladia Pringsheimii* by Thaxter (33) and by von Minden (26) and has also been observed by the writer. It is a specific character within the genus *Monoblepharis* and may likewise be one in the genus *Blastocladia*. Furthermore, this condition in any case is not indicative of parthenogenesis, because in *Monoblepharis polymorpha* Cornu it is the fertilized oöspores that germinate outside of the oögonia.

Another hypothesis concerning sexuality in the genus is that the delicate hair-like filaments present in *B. Pringsheimii* represent degenerate non-functioning antheridial branches. Little credence seems to have been given to this supposition, and since the hair-like filaments are a specific character in only two species of *Blastocladia*, its incorrectness is obvious. These hair-like outgrowths are very slender and attain a diameter of 6 to 8 μ . They are very long and in mature plants often exceed the length of the basal portion by two or three times. The hairs arise from bulbous outgrowths on any upper portion of the basal stalk or from any of its several lobes.

In contrast to these, the antheridial filaments observed by the writer in *B. globosa* sp. nov. (Pl. XXXII, fig. 1a and fig. 4a) were easily distinguishable. They have thicker walls and greater width, attaining a diameter of 14 μ . They arise from the wall of the main stalk or its lobes. There is never any indication of a bulbous base such as is present at the bases of all of the hair-like filaments. Furthermore, the antheridial branches are slightly swollen at the apical ends and do not terminate in sharp fine points. They are also shorter than the hairs, and though they do extend beyond the lengths reached by the large sporangia and oögonia, yet they never reach the extreme lengths of the slender filaments.

Nor are the antheridial branches to be confused with the branching arms of the main stalk which are frequently found in the larger plants of such a species as *B. Pringsheimii*. The walls of the lobed portions are roughened and scaly like the walls of the basal stalk itself, whereas the antheridial branches have thin, smooth walls. The lobes arise from the main stalk, appearing at first as large, swollen, bud-like processes and always have the characteristic wall and the large diameter.

The antheridial branches observed by the writer were found on two plants of *B. globosa* sp. nov. The limited number of findings establishes the fact that they are not common, since hundreds of plants of the genus have been observed carefully by the writer, and since it has been often studied by other botanists. They cannot be confused with any of the other morphological structures that have just been described. There is definite evidence for believing that they are true antheridial branches, since at the end of one of them a definite septum cut off the curved, apical portion which was undoubtedly a true antheridium (Pl. XXXII, fig. 4 a).

The process of fertilization was not observed nor was there any definite indication of how it might be effected. There are two possible ways in which it may take place, either by the passage of the gametes directly from the antheridium into the oögonium, or by means of motile male gametes. The fact that the antheridial branches extended far beyond the basal cell bearing the sessile oögonia seems to indicate that it must be effected by means of antherozoids. However, in making the microscopic mounts in which these branches were detected, the branches may have been loosened from their

attachment to the oögonia. In one instance, which could not be studied clearly because of the density of the mount, it did seem as though there was an actual attachment and tube formation at the apex of an oögonium. However, that point could not be established with any degree of certainty.

It has already been suggested by Thaxter (33) and others, that antherozoids may be formed and that, if they exist, the relationship between *Blastocladia* and *Monoblepharis* would be strengthened. Such an hypothesis, if true, would lend support to the evidence already at hand on other morphological grounds for considering these two genera to be closely related.

In spite of the observation just recorded of the existence of a true antheridium, it still remains to be proved whether fertilization actually takes place, and if so how it is accomplished. It is quite evident from the scarcity of antheridia that countless numbers of oögonia develop parthenospores. It is highly probable that what have hitherto been called "resting spores" are true oöspores. Von Minden (26), Thaxter (33), Reinsch (28) and the writer have observed germination of such spores of *B. Pringsheimii*. Since it is extremely uncommon to find oöspores of any water molds germinating even in cases where fertilization is a well known process, it is highly probable that those found germinating in *Blastocladia* were functioning merely as parthenospores. However, the significance of the finding of a true antheridium is none the less important, for it establishes sexuality in one of the families of water molds in which it was not known to exist, and indicates the likelihood that it may later be found in other species of the family.

There are other interesting structures to be considered in *B. Pringsheimii*, namely, certain slender secondary branches which bear sporangia as well as hair-like filaments (Pl. XXXIII, figs. 8 and 9). Von Minden (26, fig. 17) figures objects which he considered young plants, but which the writer has seen connected at their bases to the thallus of the main plants. He does not figure the rhizoids which are so conspicuous a part of the young plants of *B. Pringsheimii*, and it is to be suspected that he mistook for young plants the structures about to be described. His wording is "young plants with hairs." Such a misinterpretation could easily occur, as these branches are brittle at their bases and break away readily from the primary branches even when more than ordinary care is exercised in making a microscopic mount. Doubtless von Minden saw them in this detached condition. The writer satisfied herself that they grew directly from the large lobes and must be considered an integral part of the plant. Their smaller but typical sporangia and branching hairs were observed on the sides and at the ends of slender filaments forming a dense fringe extending far beyond the tips of the very large primary sporangia. The fringe was so dense and so thick that it gave the impression of countless small plants growing parasitically on the unusually large basal stalks. After considerable attention had been paid to these branches, it finally became clear that

they arose from the thick-walled lobes of the basal cell and were a definite part of the plant. There were many hair-like filaments extending far beyond even the tips of the last-formed sporangia (figs. 8 and 9), so that the aggregation of the main plant and all its attached parts formed indeed a plant of extraordinary size, even for *B. Pringsheimii*.

Another point of unusual interest is the process of zoospore formation in *B. Pringsheimii*, which differs in several respects from the process as it is known in the genus *Saprolegnia* (13, 14). Since the early stages have never been described for the genus *Blastocladia*, the initial steps in the process will be given here. The protoplasm in the sporangium is at first coarsely granular (Pl. XXXIII, fig. 8 a). It soon becomes finely granular and more homogeneous, and is then highly refractive. Very soon vacuoles appear near the sporangium wall giving the wall the appearance of being deeply and regularly pitted (fig. 10). The number of vacuoles is approximately the same as the number of zoospores that will eventually be produced in the sporangium. The vacuoles shift quickly, coalesce and cause the protoplasm to occupy the central portion of the sporangium. It then appears as an irregular mass having a wavy margin (fig. 11). The vacuoles continue to cut into this mass of protoplasm leaving several large, irregularly-shaped lumps (fig. 12). The protoplasm then expands and fills the sporangium. At this time there are numerous small vacuoles but these appear to affect the individual spore initials rather than the protoplasm as a whole.

During the early stages when the vacuoles are rapidly dividing the protoplasm into small units, the exit-papilla becomes visible (fig. 11). It looks very much like a small plug extending downward into the sporangium from the apex. The exact composition or function of the papilla in mature sporangia is not fully understood, though it is clear that it plays some rôle in the opening of the sporangium at the time of, or immediately preceding, the escape of the zoospores. Before the sporangium opens the papilla is very large and conspicuous and is highly refractive (fig. 8 a).

The later processes of spore formation have been described by Thaxter (33) and von Minden (26) and will not be repeated here.

Finally, a few remarks on the so-called "resting spores" may be pertinent. One continually finds the term "resting spore" used in connection with oöspore-like bodies found in the Leptomitales and Blastocladales. It was at first apparently applied to definite structures which were poorly understood, but it continues to be used in the literature. Bodies which are abundantly produced in *Apodachlya* spp. and in *Blastocladia* spp. come under this category. Those that have been observed in *Allomyces* (Butler, 5, p. 364), although better studied, have also been called by that name. The resting spores, however, seem to be more or less comparable to oöspores known in other genera of the Oömycetes, particularly to the parthenospores. The complexity of the wall with its three definite layers seems to suggest something other than what is meant by the vague term "resting spore." Such special-

ization of structure strongly indicates the typical oögonia well known in other genera. Furthermore, Coker (7) has demonstrated, though not beyond question, that what have been called resting spores in the genus *Apodachlya* are true oögonia. To this must be added the evidence presented in this paper for believing that in the genus *Blastocladia* these structures also are true oögonia.

The resting sporangia found in *Araiospora* and in *Rhipidium* are not to be confused with the resting spores mentioned above. They are apparently rightly named, as they have only a single wall, and are comparable to the usual zoösporangia found in these genera. Sporangia that are comparable to those resting sporangia described in detail by von Minden for *Rhipidium europaeum* were also found by the writer in *Rhipidium europaeum* forma *attenuata* f. nov. When stained with chloriodid of zinc the wall turns a deep orange-red and can be distinctly seen to consist of one layer only.

TAXONOMY²

ORDER: BLASTOCLADIALES Petersen

Mycelium composed of a large basal thallus cell attached by rhizoids, simple or bearing branches above; filamentous in *Gonapodya*. Zoöspores usually uniciliate. Oögonia containing each a single oöspore. Antheridia known only in one species. Membranes not turning blue with chloriodid of zinc, with the exception of *Mindeniella*.

FAMILY: BLASTOCLADIACEAE Petersen

Characters of the family the same as those of the order.

Differs from the Leptomitales, Saprolegniales and Peronosporales in the presence of non-cellulose membranes, unciliated zoöspores and absence of constrictions; and from the Monoblepharidales in the enlarged basal cell, the manner in which the reproductive organs are borne, elongate zoösporangia, and perhaps in lack of motile antherozoids.

ORDER: LEPTOMITALES ord. nov.

Mycelium divided into pseudo-cells by cellulose deposits which are accompanied by constrictions. Zoösporangia broadly elliptical to oval. Zoöspores commonly monoplanetic, kidney-shaped, mostly biciliate. Oögonia with one oöspore, differentiated into oöplasm and periplasm during development. Antheridia provided with a fertilization tube known in most of the genera. Membranes turning blue with chloriodid of zinc.

² Of the twenty-three species known and included in this monograph, twelve have been collected in the vicinity of Ann Arbor, Michigan, during the years 1923, 1924 and 1925.

FAMILY: LEPTOMITACEAE Schröter

Characters of the family the same as those of the order.

Differs from the Saprolegniales in presence of constrictions and of oogonia containing single oöspores; from the Peronosporales in the presence of constrictions as well as in the absence of conidia; and from the Blastocladias, in the presence of cellulose membranes, biciliated zoöspores and oogonia with oöplasm and periplasm.

FAMILY: BLASTOCLADIACEAE

KEY TO GENERA AND SPECIES OF BLASTOCLADIACEAE

1. Plant consisting entirely of filamentous, segmented, branched hyphae arising from a common base. GONAPODYA
2. Sporangia $130-250 \times 22 \mu$, mycelium constricted into segments throughout. *G. siliquaeformis*
2. Sporangia $20-60 \times 12-30 \mu$, constrictions in mycelium somewhat sparse. *G. polymorpha*
1. Plant with mycelium of basal portion greatly enlarged. 2
2. Sporangia and oogonia supported on slender, short pedicels, oogonial wall spiny. MINDENIELLA
M. spinospora
2. Sporangia sessile on basal portion or its branches. 3
3. Plant regularly septate, sporangia ovoid with several exit-papillae. ALLOMYCES
4. Constrictions accompanying the septa. *A. arbuscula*
4. Constrictions not accompanying the septa. *A. arbuscula*
forma *dichotoma*
3. Plant non-septate, sporangia cylindrical, with one apical exit-papilla. BLASTOCLADIA
4. Basal portion cylindrical, $14-50 \mu$ wide, copiously branched in a sub-dichotomous manner. 5
5. Wall of basal cell smooth. 6
6. Plant small, sporangia $30 \times 15 \mu$ *B. ramosa* *
6. Plant larger, sporangia $84-180 \times 20-30 \mu$ *B. gracilis*
5. Wall of basal cell sculptured with brownish markings. *B. tenuis*
4. Basal portion not cylindrical, or if so, swollen at the upper end. 5
5. Basal portion globose, sporangia broadly elliptical. *B. globosa*
5. Basal portion large, variously shaped, not globose. 6
6. Sporangia proliferating. *B. prolifera*
6. Sporangia not proliferating. 7
7. Sporangia arising thickly and irregularly from any portion of the basal cell, sterile hair-like filaments present. *B. Pringsheimii*
7. Sporangia arranged in a subracemose to sub-dichotomous fashion from the branches of the basal cell. *B. rostrata*

GENUS: *BLASTOCLADIA* Reinsch (Emend.), Jahrb. Wiss.
Bot. 9: 298. 1878

Plant consisting of a single large thalloid cell which is branched or unbranched, and possesses rhizoids. Zoösporangia sessile on the basal pseudo-cell, disappearing soon after the discharge of the spores and leaving a scar at place of attachment, furnished with an apical exit-papilla. Zoöspores large, not emerging in a vesicle, cilia one to three, usually one; monoplanetic. Oögonia sessile like the sporangia, with three walls, the middle one thick, perforated, colored. Antheridia known in one species.

The discovery of antheridia, even in one species, is strong evidence that the so-called "resting spores" of former authors are to be considered oögonia containing either an oöspore or a parthenospore.

BLASTOCLADIA PRINGSHEIMII Reinsch (Emend.), Jahrb. Wiss. Bot. 11: 298.
Pl. 16, figs. 1-10. 1878

Bot. Gaz. 21: 45. Pl. 5, figs. 1-3.—Ann. Mycol. 8: 532. fig. 10.—
Kryptogamenfl. Mark Brandenburg 5: 603, fig. 13, *a-d*.—Mycol. Untersuch.
2: 189. Pl. 4, figs. 25-33; text figs. 15, 16, 17.

Vegetative plant attached to substratum by means of numerous stout, branching rhizoids; variable in size and shape, 250-1000 μ high $\times$ 30-400 μ wide. Basal portion either cylindrical with swollen dome-shaped top, irregularly branched with large lobes or branches which are swollen at their ends, or merely a greatly enlarged and somewhat elongated swollen unbranched cell. Wall thick, 8-10 μ , laminate or rough-scaly at the lower outer surface; protoplasmic contents brownish in color, including numerous, conspicuous oil droplets. Hair-like sterile filaments with bulbous base, simple or branched, arising from any portion of the basal cell or from any of its branches, and measuring 200-600 $\times$ 5-6 μ .

Asexual reproduction by means of zoösporangia, sessile, arising thickly from any portion of the basal cell or from its branches, long-cylindrical, 150-350 $\times$ 30-45 μ , thin-walled, quickly disintegrating after the emergence of the zoöspores, leaving a circular scar, exit-papilla present. Zoöspores numerous, swimming directly from the sporangium without the formation of an external vesicle membrane, 12-14 μ in diameter, provided with one, two, or three cilia, monoplanetic. Additional secondary sporangia formed on specialized slender branches which arise from the primary lobes, zoösporangia similar in every respect except in size to the primary sporangia, the last-formed being smaller, 44-100 $\times$ 20-26 μ .

Sexual reproduction by means of oögonia and antheridia. Oögonia sessile, elliptical to oval, 50-70 $\times$ 30-50 μ : oögonial wall smooth, thin. Oöspore single, filling the oögonium, exospore smooth, tough, definitely and regularly punctate, inner wall thin. Oöspore germinating after emerging from the oögonial wall through a crack in the oöspore membrane. Fertilization not observed.

On submerged decaying fruits; apples, crabapples, rose, and *Crataegus*, in stagnant water. March to December, Ann Arbor, Michigan. On decaying fruits, twigs, etc. Hamburg and Breslau (von Minden); on decaying apples in water, Germany (Reinsch); on apples and branches of

ash, Denmark (Petersen); on apples and other decaying vegetable matter, Massachusetts and Maine (Thaxter).

This species is a common one in Michigan and has been studied by the writer from abundant material. The branches bearing the specialized secondary branches and sporangia have not been reported previously and are described in detail in the morphological part of the paper.

Petersen (27, p. 533) remarks upon the fact that the zoöspores were seldom seen escaping from the sporangia. The writer has watched the escape of a great many zoöspores and their exit appears to be dependent on temperature, since they swarm out when the temperature is considerably lower than room temperature. They swim actively for a few minutes before coming to rest and encysting. A great many of them fragment as soon as they leave the sporangia or even before, and give the appearance of small spores due to the accumulation of the coarsely granular protoplasm that is contained in them. The spores are large, 12–14 μ , rarely 20 μ , in size, and are therefore easily distinguished. The number of cilia as determined by the use of iodine vapor seem to be mostly two, but as already pointed out by Thaxter (33, p. 48), there are cases where only one cilium can be made out.

BLASTOCLADIA GLOBOSA SP. NOV. (Pl. XXXII)

Vegetative plant attached to the substratum by means of a few stout, branching rhizoids. Basal portion globose to sub-globose, 150–350 $\times$ 150–180 μ , rarely with large irregular branches or lobes with swollen apical ends. Wall *brittle*, thick, laminate. Slender sterile hair-like filaments sometimes present.

Asexual reproduction by means of zoösporangia, sessile, arising thickly from any portion of the basal cell, broadly cylindrical, 75–150 $\times$ 20–60 μ , thin-walled. Zoöspores numerous, biciliate, 12–14 μ , kidney-shaped to oval when swimming.

Sexual reproduction by means of oögonia and antheridia. Oögonia sessile, oval to subpyriform, 60–70 $\times$ 35–50 μ , with truncate base, readily breaking away from the basal cell, leaving a prominent scar. Germination preceded by the slipping out of the oöspore from the oögonial wall. Antheridial branches arising directly from the basal portion or from its branches, about 300 $\times$ 14 μ . Antheridium curved, cylindrical, borne terminally at the end of an antheridial branch from which it is cut off by a septum. Fertilization not observed.

On decaying submerged crabapple and *Crataegus* in shallow pool in greenhouse, Botanical Garden. Type collected by Bessie B. Kanouse near Ann Arbor, Michigan. October.

This plant was found fairly abundantly in collections made in the fall of 1925 in a shallow pool in the Botanical Garden greenhouse. It is related to *B. Pringsheimii* but differs from it by its pronounced globose basal portion, by its brittle wall, and by its stouter rhizoids which are likewise fewer in number, and by the shorter and broader sporangia. It was not uncommon in making microscopic mounts of this fungus to break the basal portion into

several parts due to the extremely brittle nature of the wall. It was in this species that the antheridial filaments and antheridium were observed, the account of which appears in the morphological part of this paper.

BLASTOCLADIA ROSTRATA von Minden, Kryptogamenfl. Mark Brandenburg
5: 604. 1912

Mycol. Untersuch. 2: 195. Pl. 4, figs. 34-35; text figs. 18-19.

Vegetative plant attached to substratum by rhizoids. Plant 500-1500 μ high. Basal portion more or less broadly cylindrical, branched dichotomously or pseudo-dichotomously, more or less irregularly, sometimes copiously, branches not swollen at apices; wall neither brittle nor laminate; protoplasm tinged brownish. Sterile threads lacking.

Asexual reproduction by means of zoösporangia which are sessile, cylindrical to subfusiform, on elongated branches, mostly spirally arranged, in dwarf plants tending to form small groups of two's and three's, 70-100 $\times$ 20-30 μ .

Sexual reproduction by means of oögonia which are broadly sessile, ellipsoid, averaging 40 $\times$ 25 μ , also up to 53 $\times$ 30 μ , greatest width in the middle, apex beaked above the rounded end; germination preceded by the slipping out of the oöspores from the oögonium; wall very thick and very definitely punctate. Antheridia unknown.

On apple and Crataegus fruits submerged in ditch, Ann Arbor, Michigan, October; also on fruits and twigs in water, Germany (von Minden).

According to von Minden (l.c.) the "resting spores" are often found in large numbers with but few or no sporangia; at times, however, only the sporangia are present or found in excess, all of which indicates the dependence of these modes of reproduction on external conditions. Von Minden points out that this species differs from *B. Pringsheimii* in the character of the mycelium and in the falling out of the "resting spores" from the outer wall. When mature, the wall layers of these spores separate in such a way that the cell with its endospore, which is rough-warty and tough, falls out of the smooth exospore through a transverse crack.

In spite of the very apparent identity of the Michigan plant with this species in other respects, our specimens differed by the size of the sporangia, which measure 120-200 $\times$ 25-30 μ .

BLASTOCLADIA PROLIFERA von Minden, Kryptogamenfl. Mark Brandenburg
5: 606. 1912

Mycol. Untersuch. 2: 213, text figs. 20, 21, and 22, on p. 200.

Vegetative plant attached to substratum by means of rhizoids; basal portion 170 $\times$ 80 μ , mostly broadly cylindrical, terminated by more or less numerous, irregularly arranged branches.

Asexual reproduction by means of zoösporangia borne terminally and in abundance at the ends of the branches; sporangia sessile on a broad base, cylindrical, often curved, somewhat narrowed at the apex; exit-papilla present, 80-150 $\times$ 15-30 μ ; proliferating by successive sporangia in a nesting fashion often up to five times.

Sexual reproduction not observed.

On submerged plant substrata, Germany (von Minden).
Adapted from von Minden (l.c.).

BLASTOCLADIA RAMOSA Thaxter, Bot. Gaz. 21: 50. Pl. 3, figs. 14-16. 1896

Kryptogamenfl. Mark Brandenburg 5: 605.—Mycol. Untersuch. 2: 212.
Pl. 5, figs. 36-37.

Vegetative plant attached to substratum by means of rhizoids. Basal portion nearly cylindrical, 14-20 μ in diameter, copiously and irregularly or subdichotomously branched. Whole plant varying from 260-600 μ in diameter.

Asexual reproduction by means of zoösporangia borne terminally or subterminally on the branchlets. Sporangia 30 $\times$ 15 μ , broadly oval, bluntly pointed.

Sexual reproduction by means of oogonia borne terminally or subterminally on the branchlets, 30 $\times$ 11 μ , terminally bluntly rounded, gradually narrowed toward the truncate base. Antheridia unknown.

On submerged sticks in a sphagnum bog, Maine (Thaxter); on decaying fruits, Germany (von Minden).

Adapted from Thaxter (l.c.).

BLASTOCLADIA GRACILIS SP. NOV. (Pl. XXXIII, figs. 14-16)

Blastocladia ramosa var. *luxurians* Kanouse, Michigan Acad. Sci. Arts and Letters 5: 105, Pl. 1, fig. 1.

Vegetative plant attached to substratum by means of scanty rhizoids. Basal portion slender, cylindrical, 20-50 μ in diameter, branched in a racemose to subdichotomous manner. Whole plant 600-850 μ long. Wall thin, smooth, hyaline, 2.5-4 μ thick. Protoplasm of basal portion finely granular, hyaline. Sterile filaments lacking.

Asexual reproduction by means of zoösporangia which in the mature plants are arranged in a racemose to corymb-like fashion, long-cylindrical, 84-180 $\times$ 20-30 μ , thin-walled, provided with a prominent exit-papilla.

Sexual reproduction by means of oogonia borne on branches in a manner similar to that in which the zoösporangia are borne, oval to subglobose, with truncate base, 50-60 $\times$ 28-36 μ . Outer wall of oöspore finely and regularly punctate. Antheridia not observed.

On submerged decaying apples in stagnant lake water. March and October. Type collected near Ann Arbor, Michigan, by Bessie B. Kanouse.

This is a rare fungus, and a beautiful, trim little plant. It was found in limited quantities growing with *Rhipidium europaeum*, and again with *B. Pringsheimii*. It is conspicuous because of the slender, thin, smooth, hyaline basal portion and the hyaline protoplasm. It is also distinguishable from *B. ramosa* by its relatively sparse branching, by the scanty production of rhizoids and by the much larger size of the sporangia and oogonia.

Thaxter figures a plant (33, Pl. 3, fig. 6) which he considered *Blastocladia Pringsheimii* but a "small plant, the habit and form of resting spores unusual." It is highly probable that it is identical with *B. gracilis*. Likewise Petersen (27, fig. 10 d) shows a slender branched plant which he called a

form of *B. Pringsheimii*. In all probability it also is *B. gracilis*. In neither of the texts cited above is an adequate description given of the plants upon which to make a definite conclusion concerning their identity.

BLASTOCLADIA TENUIS SP. NOV. (Pl. XXXIII, figs. 5-7)

Vegetative plant attached to the substratum by means of a few delicate rhizoids. Basal portion cylindrical $300-1000 \times 30-60 \mu$, if at all branched then only once or twice near the middle, wall thin, $3-4 \mu$, *very brittle, sculptured throughout with ridged markings, golden brown in color.*

Asexual reproduction by means of zoösporangia borne terminally at the apices of the basal portion or its branches, $27 \times 11 \mu$, not abundant.

Sexual reproduction by means of oögonia borne terminally, singly or irregularly clustered at the ends of the basal portion, $24-40 \times 22-24 \mu$. Antheridia not observed.

On decaying submerged rose and Crataegus fruits in stagnant water. November and May. Type collected by Bessie B. Kanouse near Ann Arbor, Michigan.

These plants were found growing in tufts on the surface of fruits in stagnant water. They were conspicuous because of the coloring and sculpturing of the wall, the delicate and slender cell, the scanty branching and the small numbers of reproductive organs. Many of the plants were seen before it was possible even to locate them in a genus. The wall markings are unlike anything known in other blastocladias and are not to be attributed to parasites or parasitic water molds growing in the cell, as this point was considered with special care. It differs from all other species by its small reproductive organs.

GENUS: **MINDENIELLA** GEN. NOV. (In honor of M. von Minden.)

Plant consisting of a large, unbranched thalloid cell attached by rhizoids. The reproductive organs borne on slender short pedicels; pseudo-septa immediately below the sporangia or oögonia. Zoösporangia large, thin-walled, pyriform, the broadest part near the apex. Oögonia spherical with spiny walls; oöspore single, with colored exospore, nearly filling the oögonium. Antheridia not observed. Membranes turning blue with chloriodid of zinc. The type-species is *M. spinospora*.

MINDENIELLA SPINOSPORA SP. NOV. (Pl. XXXIV)

Vegetative plant attached to substratum by means of penetrating rhizoids forming with the rhizoids a single large cell, elongate-clavate, $200-600 \times 100-150 \mu$; apex rounded into a dome from the surface of which arise the sporangia-bearing pedicels. Wall very thick, laminate, scaly near base from the shredding of the wall. Protoplasm dark-colored, coarsely granular, containing numerous oil droplets. Pseudo-septa formed of cellulose present in upper end of each pedicel.

Asexual reproduction by means of zoösporangia which are borne singly at the apices of the densely disposed pedicels. Zoösporangia large, $70-160 \times 60-75 \mu$, broadest near the apex, pyriform to oval-elliptical, thin-walled, with or without a single row of four to eight spines surrounding the exit-

papilla of the sporangium. Sporangia cut off completely from the vegetative cell by a cellulin plug. Zoöspore formation as in the genus *Saprolegnia*.

Sexual reproduction by means of oögonia which are formed on pedicels like those bearing sporangia, easily broken off at maturity with pedicel attached; appearing after the zoösporangia. Oögonial wall thick, brittle, *entire surface spiny*. Spines 14–30 μ long. Wall and spines staining faintly blue with chloriodid of zinc. Oöspore single, composed of finely granular viscous protoplasm; exospore smooth, light golden brown in color. Antheridia unknown.

On decaying *Crataegus* fruits and on apple submerged in stagnant shore water of lake. October. Type collected by Bessie B. Kanouse near Ann Arbor, Michigan.

This is evidently a rare plant as it was found only in two collections made during September and October 1925. It grew in tufts most effectively concealed by *Rhipidium americanum*. First observations give the impression of a species of *Blastocladia* because of the shape and size of the thick-walled swollen basal cell. The pedicels are not easily observed, covered as they are by the crowded sporangia, so that the sporangia seem at first glance to be sessile. When stained with chloriodid of zinc the walls of the entire plant with the exception of the cellulin plugs turn a deep "litho purple" (Ridg).³ On one occasion *Blastocladia Pringsheimii*, *Rhipidium americanum* and *Mindeniella spinospora* were found together on the same slide to which the chloriodid of zinc was applied so that the striking contrasts in the reactions could be easily and simultaneously observed. The first-named fungus turned a yellow brown, while the two others stained blue. The spiny oögonial wall is peculiar to this genus alone and serves to distinguish it easily.

GENUS: ALLOMYCES⁴ Butler, Annals Bot. 25: 1023, figs. 1–18. 1911

Plant consisting of differentiated basal axis with rhizoids and fertile filaments, somewhat constricted into segments by perforated or more or less complete cross walls. Filaments branched sympodially or dichotomously. Zoösporangia borne terminally on filaments, single or catenulate, ovoid, with several exit-papillae. Zoöspores emerging singly without formation of vesicle, large, oblong or elliptical, cilia one to three, usually one, amoeboid at first, monoplanetic. Resting spores (oögonia?) terminal on filaments, ovoid, wall of three layers, the middle one thick, colored, formed within terminal cell of filament and set free by its rupture or sometimes completely filling it and then thrown off with the terminal cell; germination by zoöspores.

ALLOMYCES ARBUSCULA Butler, Annals Bot. 25: 1023, figs. 1–18 on p. 1035. 1911

Blastocladia strangulata Barrett. Bot. Gaz. 54: 353. Pls. 18–20.

Allomyces strangulata (Barrett) von Minden.

³ "Ridg." used here and elsewhere in this paper refers to Ridgway's Color Standards and Color Nomenclature.

⁴ ALLOMYCES MONILIFORMIS n. sp. Coker and Braxton was published after this paper had gone to press. Jour. Elisha Mitch. Soc. 42:139. 1926.

Vegetative plant filamentous attached to substratum by rhizoids. Basal cells $100-200 \times 40-100 \mu$, one or more times dichotomously or sub-umbellately branched above, fertile branches arising in groups of two or more from the end segments of the basal body, more or less definite constrictions and perforated pseudo-septa at points of branching, cells up to $250 \times 15-25 \mu$, whole plant $200-2000 \mu$ high.

Asexual reproduction by means of zoösporangia which terminate a branch singly or in chains, broadly elliptical ovoid to sub-globose, $40-70 \times 30-52 \mu$, ends bluntly rounded or truncate, with one to four exit-papillae; zoöspores large, $12 \times 6 \mu$, with one cilium, rarely two to three.

"Resting spores"! (oögonia) single, terminal, oval to nearly oval, truncate below, rounded above; wall of three layers, the outer and inner thin, the middle pitted or perforated, orange to brown; on germination giving rise to zoöspores.

On dead flies, India (Butler); on aphid in water and from garden soil, New York and Illinois (Barrett).

This description is adapted from Butler (l.c.) and from Barrett (l.c.). Since this genus was not represented in the cultures at Ann Arbor, although it doubtless exists here, it seems best to follow von Minden's synonymy. Fitzpatrick (12) and Coker (7) may or may not be correct in assuming the identity of Barrett's and Coker and Grant's plants. Apparently, the two need to be studied side by side in the living condition. Meanwhile, using the facts set forth in their descriptions, it would appear that Coker and Grant's plant is at least a form, and hence should be recognized as follows:

Forma **dichotoma** (Coker and Grant) comb. nov. (*Septocladia dichotoma* Coker and Grant). This form is definitely stated to have no constrictions. It was obtained by Grant on a bone lying in the water, North Carolina.

GENUS: GONAPODYA Fisher, Rabenh. Krypt. Fl. 1, 4: 382. 1892

Plant consisting of a filamentous mycelium, segmented by pseudo-septa into short, broad segments, usually though not always accompanied by definite constrictions. Zoösporangia fusiform-lanceolate to elliptic-ovate, proliferating. Zoöspores mostly uniciliate. Sexual reproduction unknown. Membranes not turning blue with chloriodid of zinc.

GONAPODYA POLYMORPHA Thaxter, Bot. Gaz. 20: 481, Pl. 31, figs. 11-16. 1895

Kryptogamenfl. Mark Brandenburg 5: 579.—Ann. Mycol. 8: 534, text figs. 12, 13 and 14.

Vegetative plant composed of segmented hyphae arising directly from the substratum or more often the segmented portion consisting of short, oval or irregular segments confined to tufts of branchlets borne subumbellately on the ends of more or less slender elongate hyphae, in which the segmentation is indistinct or obsolete; segmentation frequently ill-defined or obsolete throughout the whole vegetative body; pseudo-septa not always accompanied by constrictions. Hyphae $200-1000 \mu$ long.

Asexual reproduction by means of zoösporangia, terminal and solitary or sometimes several arising from a single segment, once to several times proliferous, the hyphae sometimes traversing and growing beyond the empty

sporangia; of variable form, long-oval, tapering rather abruptly to the blunt tip, $20-60 \times 12-30 \mu$. Zoöspores very variable in size and number, resembling those belonging to *G. siliquaeformis*, uniciliate (sometimes seeming to be biciliate).

Sexual reproduction unknown.

On submerged twigs and other vegetable matter, Massachusetts and Maine (Thaxter); on fir and spruce found in the mucilage which is formed by the destruction of the resin, Denmark (Petersen).

No oöspores have been found in actual connection with the zoösporic plants, but Thaxter described and figured some which might have belonged to this fungus. They were about 54μ in diameter and were said to resemble the oöspores of *Rhipidium*; to have a laminate, refractive wall 18μ thick; and to have a rounded usually persistent antheridium attached.

Adapted from Thaxter.

GONAPODYA SILIQUAEFORMIS (Reinsch) Thaxter, Bot. Gaz. 20: 480, Pl. 31, figs. 6-10. 1895

Saprolegnia siliquaeformis Reinsch, Jahr. Wiss. Bot. 11: 293. Pl. 15, figs. 12-13.—*Gonapodya prolifera* Fischer, Rabenh. Krypt. Fl. 1, 4: 382.—Schröter, Engler-Prantl Nat. Pfl. Fam. 1, 107.—*Monoblepharis prolifera* Cornu, Bull. Soc. Bot. France 18: 59, and Ann. Sci. Nat. Bot. sér 5: 16.—Kryptogamenfl. Mark Brandenburg, 5: 577. Fig. 12, p. 580.—Ann. Mycol. 8: 533, text fig. 11.—Mycol. Untersuch. 2: Pl. 6, fig. 6.

Vegetative plant not attached by means of rhizoids, entirely filamentous, rather stout, $250-500 \mu$ long, copiously and successively subumbellately branched; bush-like, branches diverging from a common base or sometimes forming a tangled network. Hyphae more or less regularly divided into short elliptical to long clavate segments, normally $25 \times 14 \mu$, varying greatly; segments immediately below the sporangia short, elliptical or almost sub-spherical.

Asexual reproduction by means of zoösporangia borne sessile on very long hyphae as the terminal cells of the branches, or umbellately arranged, sometimes separated by a clearly defined constriction. Sporangia long pod-shaped, inflated below, sometimes the very elongate distal portion tapering gradually to a blunt apex, $130-250 \times 22-30 \mu$. Sporangia often once to three times proliferous. Zoöspores numerous, more than fifty, hyaline, with conspicuous nuclei and weak granular forward end, one backward moving cilium.

Sexual reproduction not definitely known.

On apple in water, Ann Arbor, Michigan, May; on decaying apples in water, Massachusetts and Maine (Thaxter); Germany (Reinsch); on fruits lying in water, Germany (von Minden); France (Cornu); on submerged branches of different sorts of trees, Denmark (Petersen).

Thaxter (l.c.) states that this is apparently rare in America as he found it only once. The writer also found it only in limited quantity in a single collection.

Concerning this species, Petersen states that the "membranes contain no cellulose."

Oöspores were described by Cornu for *Monoblepharis prolifera* Cornu, a plant which may have been this species. Thaxter thinks that what Cornu really saw were the proliferating sporangia nested within one another, giving the appearance of thick-walled oöspores.

Adapted from von Minden and Thaxter.

DESCRIPTION OF PLATES

The drawings were outlined under low magnification using 16 mm. objective and no. 10 ocular. The details were then drawn in, using 3 mm. objective and no. 15 ocular. All the large figures of the plates are drawn to scale except figure 27 which, in comparison, is half size.

PLATE XXXII

Blastocladia globosa sp. nov.

FIG. 1. Plant with the uppermost portion broken away due to the brittleness of the wall. (a) antheridial branches.

FIG. 2. Plant bearing oögonia. (a) empty zoösporangia, (b) detached oögonia.

FIG. 3. Typical plant bearing zoösporangia.

FIG. 4. Plant with a rather unusual number of branches, which shows: (a) antheridium separated from the antheridial branch by a true septum; (b) slender hair-like filaments; (c) zoösporangia; (d) oögonia.

PLATE XXXIII

Figures 5-7, *Blastocladia tenuis* sp. nov.; figures 6-13, *Blastocladia Pringsheimii*; figures 14-16, *Blastocladia gracilis* sp. nov.

FIG. 5. Plant showing characteristic sculpturing of the wall of the basal cell. Note the oögonium at the apex.

FIG. 6. Portion of a plant bearing (a) a zoösporangium, (b) an oögonium.

FIG. 7. Apical portion of a plant showing details of the sculpturing of the wall.

FIGS. 8, 9. Specialized branches bearing zoösporangia and hair-like filaments characteristic of *B. Pringsheimii*. (a) zoösporangium like that formed directly on the basal cell except that it is smaller in size, zoöspores nearly mature; (b) part of basal cell to which these branches are attached.

FIG. 10. Zoösporangium showing vacuolization of the protoplasm in an early stage of spore formation. The vacuoles are very regularly placed.

FIG. 11. Sporangium showing the appearance of the protoplasm soon after some of the vacuoles have fused. The protoplasm has drawn away from the wall and is forming into irregular lumps. The papilla is beginning to be formed.

FIG. 12. Sporangium showing a still later stage of development in which the irregular masses of protoplasm are very distinct.

FIG. 13. (a) zoöspores stained with iodine to reveal the cilia; (b) germinated spore.

FIG. 14. Plant showing (a) the typical racemose arrangement of the zoösporangia, (b) two oögonia.

FIG. 15. Enlarged zoösporangium from which the zoöspores have been discharged.

FIG. 16. Enlarged oögonium.

PLATE XXXIV

Mindeniella spinospora sp. nov.

FIG. 17. A typical plant bearing the spiny oögonia. Lamellate wall peeling off in large patches.

FIG. 18. Plant bearing both oögonia and zoösporangia.

FIG. 19. Plant bearing only large empty zoösporangia supported on short pedicels.

FIG. 20. Zoösporangia showing the attachment to the basal cell. (a) zoösporangium with numerous vacuoles in an early stage of zoöspore development; (b) zoösporangium in a later stage of development. The protoplasm is becoming more evenly distributed at the periphery of the sporangium.

FIG. 21. Zoösporangia showing the appearance of the protoplasm in various stages of the development of the zoöspores. (a) the protoplasm becoming cut up into small units which are plainly distinct from one another following the reticulate appearance seen in figure 20 b; (b) the next stage after (a), in which the protoplasm becomes nearly homogeneous and surrounds a central vacuole; (c) the appearance of the initial protoplasmic masses which become rounded up into spores; (d) zoösporangium with zoöspores about to escape. Note the two spines at the place of exit of the spores.

FIG. 22. (a) a circle of spines as they are occasionally seen surrounding the place of exit; (b) an empty zoösporangium also with two spines.

FIG. 23. Spores germinating.

FIG. 24. Mature oögonium in median section showing the dense protoplasm in the interior, the three wall layers, and the pedicel with the upper portion plugged with cellulose.

FIG. 25. Mature oögonium in surface view, the viscid protoplasm oozing out of a broken portion.

FIG. 26. An older oögonium showing the appearance of the protoplasm at that stage.



KANOUSE: WATER MOLDS



KANOUSE: WATER MOLDS



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A MONOGRAPHIC STUDY OF SPECIAL GROUPS OF THE WATER MOLDS II. LEPTOMITACEAE AND PYTHIOMORPHACEAE

BESSIE BERNICE KANOUSE

(Received for publication October 26, 1926)

FAMILY: LEPTOMITACEAE

KEY TO GENERA AND SPECIES OF THE LEPTOMITACEAE

1. Plant filamentous throughout, mycelium flaccid.....2
 2. Zoösporangia cylindrical.....3
 3. Zoösporangia formed directly from the terminal segment or from successive subterminal segments; zoöspores diplo-netic.....LEPTOMITUS
L. lacteus
 3. Zoösporangia terminal or in whorls below the segments of the hyphae.....SAPROMYCES
 4. Antheridia of androgynous origin.....*S. androgynous*
 4. Antheridia on long winding branches not associated with the oögonia.....*S. Reinschii*
 2. Zoösporangia pyriform to oval.....APODACHLYA
 3. Oögonial wall punctate.....*A. punctata*
 3. Oögonial wall not punctate.....4
 4. Segments 50-100 μ long, cylindrical.....*A. brachynema*
 4. Segments 100-200 μ long, cylindrical.....*A. pirifera*
1. Plant with greatly enlarged basal portion with filamentous branches.....2
 2. Fertile branches umbellately branched; exospore of mature oöspore formed of a single layer of cells.....ARAIOSPORA
 3. Spines on the spined sporangia arranged in one or two rows around the place of exit of the zoöspores.....*A. pulchra*
 3. Spines on the spined sporangia appearing over the entire surface of the sporangium.....*A. spinosa*
 2. Fertile branches simple, sympodially branched; exospore of the mature oöspore having an areolate appearance, not cellular..RHIPIDIUM
 3. Pseudo-cell dichotomously branched, antheridia of androgynous origin.....*R. americanum*
 3. Pseudo-cell elongate with or without apical expansion into lobes. Antheridia of diclinous origin.....4
 4. Sporangia and oögonia arranged in umbels on short sub-globose branches.....*R. parthenosporum*

4. Sporangia borne terminally on long filamentous branches. 5
5. Fertilization tube entering the oögonial wall at a region
near the apex of the oögonium. *R. Thaxteri*
5. Fertilization tube entering the oögonial wall at a region
near the base of the oögonium. *R. europaeum*

GENUS: LEPTOMITUS Agardh, Sylloge Alg. 47. 1824.—Apodya
Cornu, Ann. Sci. Nat. Bot. V, 15: 14. 1872

Mycelium filamentous, slender, copiously branched, floccose, constricted into long segments by pseudo-septa. Sporangia formed directly from terminal segments of mycelium or formed from successive subterminal segments. Zoöspores diplanetic, biciliate. Sexual reproduction unknown.

LEPTOMITUS LACTEUS (Roth) Agardh, Sylloge Alg. 47. 1824

(See von Minden, Kryptogamenfl. Mark Brandenburg 5: 582 for literature.)

Vegetative plant attached directly to substratum without rhizoids, segmented, basal segments stout, up to $48\ \mu$ thick, not easily distinguishable, constricted by evident constrictions into segments averaging $100\text{--}400 \times 10\text{--}20\ \mu$, the final ones almost ten times as long as broad, but sometimes spherical or the constrictions lacking; branching abundant, usually under a constriction, monopodial, but often appearing dichotomous near the base; one to several cellulose bodies in each segment, mostly near the constrictions.

Asexual reproduction by means of zoösporangia, cylindrical like the segments but sometimes a little broader, solitary or a few in a row. Zoöspores oval, almost $12\ \mu$ long, biciliate, cilia attached terminally, spores escaping from an opening usually at the side of the sporangium, swarming at once, sometimes diplanetic, on coming to rest developing a wall and germinating by a germ tube.

Sexual reproduction not known.

On fruits submerged in stagnant water, Ann Arbor, Michigan, December. Cosmopolitan. Known to occur in drainage from sugar factories, breweries, distilleries, slaughterhouses, city waste, etc., in stagnant and occasionally in clear waters.

The constrictions are sometimes lacking, according to von Minden (l. c.). This species was early known and described and there is an array of synonyms belonging to the older literature.

Adapted from von Minden.

GENUS: APODACHLYA Pringsheim, Ber. Deutsch. Bot. Ges. 1: 289. 1883

Mycelium filamentous, floccose, copiously branched, constricted into rather regular segments by pseudo-septa. Rhizoids lacking. Mycelial wall turns faintly blue with chloriodid of zinc. Zoösporangia pyriform or broadly oval. Zoöspores formed in small numbers, sometimes encysting at the mouth of the sporangium in an Achlya-like manner, diplanetic, biciliate. Oögonia with one oöspore which nearly fills the oögonium. Oöspore containing one large oil drop. Antheridia unknown.

APODACHLYA PIRIFERA Zopf, Nov. Acta Acad. Leop. 52: 362.

Pl. 21, figs. 1-21. 1888

Leptomitus piriferus Zopf. Die Pilze, Schenk's Handbuch 4: 299.—
Kryptogamenfl. Mark Brandenburg 5: 585, fig. 15, *a-b*, 580.

Vegetative plant woven over the surface of the substratum, lacking rhizoids, hyphae copiously branched, segmented, basal segments becoming stout, smaller toward the ends, segments long cylindrical.

Asexual reproduction by means of zoösporangia which are terminal, mostly pyriform, but also oval to fusiform, $12-24 \times 12-20 \mu$, with short terminal or sometimes laterally placed exit-papilla; sporangia becoming sympodially arranged, as many as twelve sporangia sometimes so placed. Zoöspores becoming grouped in a hollow sphere at the mouth of the sporangium immediately on emergence from the sporangium.

Sexual reproduction by means of oögonia which are mostly terminal on long branches, seldom on short segmented side branches or intercalary; spherical, with a double (?) membrane, at maturity with colorless contents and a gigantic oil drop. Antheridia unknown.

On decaying Characeae (Zopf).

Doubtless the so-called "resting spores" of Zopf (l. c.) are here, as elsewhere, composed of more than two wall layers.

Tiesenhausen (35) describes a variety of this species which he calls *Apodachlya pirifera* var. *macrosporangia* which differs chiefly in having hyphae of greater diameter and longer sporangia, measuring $37-50 \mu$ in length. On needles of *Picea excelsa* submerged in water. Switzerland.

Adapted from von Minden.

APODACHLYA PUNCTATA von Minden, Kryptogamenfl. Mark

Brandenburg 5: 586, fig. 15*c-d*. 1912

Vegetative plant woven over the surface of the substratum, rhizoids lacking, filamentous, segmented, branched.

Asexual reproduction by means of zoösporangia which are formed terminally but become sympodially arranged by the continuation of the growth of the branchlets, often in quite abundant groups, clavate, oval to pyriform or subglobose, opening usually at the side of the sporangium, sometimes near the base of the sporangium. Zoöspores never or seldom forming a hollow sphere, usually swimming directly on emerging from the sporangium.

Sexual reproduction by means of oögonia ("resting spores") formed either at the ends of long hyphae or on mostly strongly reduced segments or branches.

On decaying plant substrata, twigs, stems, etc., Breslau and Hamburg (von Minden).

Adapted from von Minden.

Von Minden remarks that the "resting spore" is similar to that of *A. pirifera* but distinct by the finely regularly punctate inner oögonial wall. Coker (7) in commenting on *A. punctata* says, "Minden's species is more than doubtful." The punctate wall of the oögonia is believed by Coker to be merely an emulsion of the protoplasmic contents of the oögonia and

not to be a definite morphological character. We have included it because of our belief that von Minden is sufficiently experienced to know what he saw.

APODACHLYA BRACHYNEMA (Hildebr.) Pringsh., Ber. Deutsch. Bot.

Ges. 1: 289. 1883

Leptomitus brachynema Hildebr., Jahrb. Wiss. Bot. 6: 261. Pl. 15, figs. 13-23.—*Apodya brachynema* (Hildebr.) Cornu, Ann. Sci. Nat. Bot. V, 15: 14.—Kryptogamenfl. Mark Brandenburg 5: 587.—The Saprolegniaceae, 173, Pl. 59, figs. 1-22.

Vegetative plant not attached to substratum by rhizoids, entirely filamentous, floccose, branching copiously, segmented by constrictions which are accompanied by pseudo-septa composed of cellulin; septa in old mycelium forming complete separation between the adjacent segments. Cellulin bodies one to several in each segment; segments $50-100 \times 8-20 \mu$, averaging $60 \times 12 \mu$, variable in size and shape, becoming longer, dumb-bell-shaped or in chains of subglobose Monilia-like cells. Branches one to several arising usually from the distal ends of segments, forming obtuse angles with the main filament. Cell membranes tardily turning faintly blue with chloriodid of zinc.

Asexual reproduction by means of zoösporangia borne terminally at the ends of long hyphae or at the ends of lateral branches which are composed of few to many long segments, subglobose, ovate-elliptic, oval to pyriform, $44-76 \times 30-44 \mu$, thin-walled. Exit-papilla not conspicuous. Zoöspores few in number, swimming directly from the sporangia without encysting near the place of exit, $12-14 \mu$, diplanetic (Coker).

Sexual reproduction by means of oögonia borne singly at the ends of short lateral branches composed of a few Monilia-like segments. Oöspores single, filling the oögonia, surrounded by three walls, the outer and inner of which are thin, the middle one thick, hyaline, of homogeneous consistency. Contents of old oöspores evenly and finely granular, disposed at the periphery of the spore together with many small, regularly placed oil droplets, the center of the spore consisting of a large vacuole and one or two large oil droplets; spherical, $20-40 \mu$. Antheridia unknown.

On submerged decaying apples and Crataegus fruits, Ann Arbor, Michigan. March, November. On immersed decaying stems, Bonn (Hildebrand); on termite ant, North Carolina (Coker); Massachusetts (Thaxter).

From the original. Pure culture studies were made by the writer. Coker's interpretation of the so-called "resting spores" being true oögonia is undoubtedly correct. The fact that three distinct walls are present surrounding a mature oöspore seems to indicate that these bodies should not be called "resting spores."

The writer has also observed the hypogenous cells that Coker has described but the latter's supposition that such cells are probably antheridia needs further elucidation or cytological proof. Attention is here called to the quite similar hypogenous cells in *Saprolegnia hypogena* de Bary, in which, as Kauffman (17) has shown, the hypogenous cell is not used directly

as an antheridium, for the antheridial filaments were found capable of originating either from the hypogenous cell or from any part of the plant. The apical extensions into the oögonium were considered non-functional. The observations of Coker raise the question whether the several hypogenous cells from which he found the protoplasm passing into the young oögonium, presumably through the perforated cellulose masses which usually exist at the constrictions, do not merely serve as feeding organs for the enlargement of the oögonium during its development. It is, of course, conceivable that these pseudo-cells might be originating sources of antheridial filaments, but as yet there is no such evidence.

Tiesenhausen (35) described a variety as *A. brachynema* var. *major* which differs chiefly in having hyphae of a greater diameter as well as sporangia of greater length. This was found in a slimy mass at the surface of the water. Switzerland.

GENUS: SAPROMYCES Fritsch, Österr. Bot. Zeitschr. 43: 420. 1893

Naegelia Reinsch, Jahrb. Wiss. Bot. 11: 298.—Naegeliella Schröter, Engler and Prantl Nat. Pfl. Fam. 1: 103.

Mycelium filamentous with a slightly differentiated basal pseudo-cell with few or no rhizoids, constricted at the pseudo-septa, branched at the apex of each successive segment to form a spreading structure, which is often extensive. Walls turn blue with chloriodid of zinc. Zoösporangia generally formed at the branching apex of each segment, or terminal, or in whorls at the constrictions, elongate, subcylindrical to clavate. Zoöspores either emerging and escaping directly or sometimes emerging into a vesicular membrane which ruptures almost at once, apparently monoplanetic, biciliate. Oögonia subglobose or pyriform, narrowed toward the pedicelled base, which is differentiated from the hyphae by a cellulose plug. Oöspore solitary, spherical with scanty periplasm. Antheridial branches of androgynous or heterogynous origin, arising distally from the segments, the portion immediately below the antheridium twisted on itself. Antheridium oblong, curved, penetrating the oögonia at the apices, indenting the walls; fertilization tube present.

A somewhat confusing inconsistency has appeared in the literature concerning the escape of the zoöspores and the presence or absence of a vesicular, extruded membrane at the time of the zoöspore formation in this genus. Petersen (27) misquotes Thaxter, while Coker (7) quotes Petersen so that the result is misleading, to say the least. Thaxter in his first paper (31) clearly states that "there was no indication of any process similar to that described by Cornu in Rhipidium, where the contents of the sporangium is said to be discharged simultaneously as a mass of zoöspores which are then set free by the rupture of a thin surrounding membrane." Turning now to Thaxter's second paper in which he discusses Sapromyces (34, p. 323), we find Thaxter reporting as follows: "The species previously described (*S. Reinschii*) has been observed by the writer in great abundance and in perfect condition since the publication of the note above mentioned, and in specimens growing under favorable conditions

the emission of the zoöspores has been seen to be similar to that of *Rhipidium* except that the membrane surrounding the emerging spore mass is ruptured almost immediately, so that all but a small number of the spores escape in the usual way through the open mouth of the sporangium."

SAPROMYCES REINSCHII (Schröter) Fritsch, Österr. Bot. Zeitschr.
43: 420. 1893

Bot. Gaz. 19: 54. Pl. 5, figs. 1-9.—Hyphomycetorum nov. gen. Reinsch, Contrib. ad Algol et Fungol 1: Pl. 14.—*Naegelia* sp. 1-2 Reinsch, Jahrb. Wiss. Bot. 11: 298. Pl. 15, figs. 1-11.—*Sapromyces dubius* Fritsch (l. c.).—Kryptogamenfl. Mark Brandenburg 5: 589, fig. 11.—Fischer, Phycomycetes, in Rabenh. Kryptogamenfl. Pilze 1, pt. 4: 377.—*Naegeliella Reinschii* Schröter, Engler and Prantl Nat. Pfl. Fam. 1: 103.—Ann. Mycol. 8: 527, fig. 4 (*b*, *c* and *d*), and fig. 5.—*Rhipidium elongatum* Cornu, Ann. Sci. Nat. Bot. V, 15: 15.

Vegetative plant attached to substratum directly without rhizoids or with only a few weakly developed ones, composed of numerous successive nearly cylindrical segments arising one to several from scarcely differentiated basal segments which measure $450 \times 10-15 \mu$ or longer, each segment producing distally one to several similar segments; constrictions between segments usually closed with cellulin plugs or more commonly without such pseudo-septa, the contents of successive segments being in direct communication with one another.

Asexual reproduction by means of zoösporangia which are borne singly or in whorls of two to six at the free end of a branch, or on the segments at the constrictions; subcylindrical to elongate-elliptical, $35-200 \times 20-25 \mu$. Zoöspores escaping through a terminal pore or the whole zoöspore mass emerging and surrounded by a vesicular membrane which is immediately ruptured, when emerging separately with the ciliated end backward, kidney-shaped, with two cilia arising from a depression at the side, apparently monoplanetic.

Sexual reproduction by means of oögonia and antheridia. Oögonia borne terminally or laterally like the sporangia and often associated with them, sub-spherical to pyriform, $26-40 \times 32-55 \mu$, becoming covered at maturity by a brown, flaky incrustation disposed transversely, and containing a single, nearly smooth, spherical oöspore, $20-30 \mu$ in diameter, with thick yellowish membrane. Antheridia borne like the oögonia but not associated with them, on long winding twisting branches irregularly cylindrical, abruptly distinguished from the antheridial branch, sometimes divided by a septum, penetrating the oögonium at its apex by a beak-like pollinodium.

On *Viscum* stems, on algae, Germany (Reinsch); on cones and twigs of *Pinus*, Maine (Thaxter); on old branches of fir, Denmark (Petersen); on pine twigs, Germany (von Minden); in North Carolina (Couch); in Switzerland (Tiesenhausen). Von Minden says that it was also found in Germany by Claussen.

Adapted from Thaxter. Coker (7) has given a detailed account of the various methods of spore discharge in this species, from which can be inferred the extreme sensitiveness of this process to external conditions.

SAPROMYCES ANDROGYNOUS Thaxter, Bot. Gaz. 21: 329. Pl. 22, figs. 16-19. 1896.—Kryptogamenfl. Mark Brandenburg 5: 591

Vegetative plant attached directly to substratum without rhizoidal outgrowths. Like *S. Reinschii* except somewhat smaller, total length 500-1000 μ .

Asexual reproduction by zoösporangia, $75 \times 26 \mu$.

Sexual reproduction by means of oögonia and antheridia. Oögonia pyriform, $35-50 \times 27-30 \mu$, sometimes encrusted by a blackish scaly deposit. Oöspores spherical, $20-26 \mu$, the thick colorless wall more or less modified by the presence of elevations which sometimes give a roughly undulate outline. Antheridial branches of androgynous origin, arising close to the base of the oögonium from the same segment, a spiral twist usually present below the antheridium which applies itself to the apex of the oögonium and is in form similar to that of *S. Reinschii*.

On submerged sticks, Massachusetts (Thaxter).

Adapted from Thaxter.

GENUS: RHIPIDIUM Cornu, Bull. Soc. Bot. France 18: 53. 1871.—Ann. Sci. Nat. Bot. V, 15: 15. 1872

Mycelium differentiated into a monstrously developed basal pseudo-cell which is distally lobed or branched, with numerous rhizoids at its base. Wall greatly thickened, turning blue with chloriodid of zinc. Branches becoming sympodially branched below the originally terminal sporangia, segmented by a constriction at their point of origin, seated on a short pedicel due to constriction. Zoöspores emerging from the sporangia in a cylindrical mass surrounded by a vesicular membrane and surmounted by a true papilla of dehiscence, biciliate, monoplanetic. Oögonia usually borne terminally on a pedicel formed by constriction, differentiated into oöplasm and periplasm, globose or subglobose; oöspore during its development connected with the oögonial wall by radial periplasmic strands, at maturity free from the oögonial wall and sculptured in an areolate manner. Antheridia usually small, applied near the base of the oögonia (at the apex in *R. Thaxteri*). Fertilization tube present and formed by the antheridium.

RHIPIDIUM EUROPAEUM (Cornu) von Minden, Kryptogamenfl. Mark Brandenburg 5: 597. Fig. 9 a-e. 1912

Rhipidium continuum and *R. interruptum* Cornu, Ann. Sci. Nat. Bot. V, 15: 15.—van Tieghem, Traité Bot. 1024, fig. 617.—Falck, Mycol. Untersuch. 2: 187. Pl. 2.—Kanouse, Mich. Acad. Sci. Arts and Lett. 5: 105. Pl. 1, fig. 2.

Vegetative plant attached to substratum by means of numerous penetrating branched rhizoids. Thallus extremely variable in shape and size; sometimes the disk-shaped basal portion with its broad lobes arises from a narrow stalk-like base, its main portion measuring up to 800 μ wide, its lobes up to 150 μ wide; more often elongated, subcylindrical to vase-shaped or with a flabelliform outline, varying between 250 and 950 μ long, and from 10-150 μ wide, lobes broad, rounded to subtruncate at apex. Wall up to 20 μ thick, outer surface often rough-scaly, turning to "litho-purple" (Ridg.) with chloriodid of zinc. Branches abundant, arising from the upper periphery of the basal portion and its lobes, sometimes from any

portion, 50–500 μ long $\times$ 7–12 μ wide, generally each branch at point of origin with pedicel-like constriction, above which a bulbous enlargement occurs, the constrictions being repeated along the filaments, or scanty, or absent, very rarely branching a second time.

Asexual reproduction by means of zoösporangia which are borne terminally and singly at ends of branches, each with a pedicel-like subsporangial constriction, branches from below the sporangia giving the effect of a sympodial arrangement, sometimes several so arranged; sporangia of two kinds, thick-walled and not collapsing after being emptied, or with thin, collapsible membranes, variable in size and shape, usually oval, broader at base, sometimes almost spherical or ellipsoid, 50–65 $\times$ 27–38 μ . Zoöspores biciliate, monoplanetic, 12–13 μ , spherical, escaping in a cylindrical vesicle which precedes in part the escape of the spore mass, and which is accompanied by the lifting off of the dehiscent papilla, spores immediately breaking through the vesicle and swimming away.

Sexual reproduction by means of oögonia and antheridia. Oögonia borne terminally on the ends of the branches, provided with suboögonial pedicel-like constrictions, globose, 50–60 μ in diameter; oögonial wall quite thick, smooth. Oöspore solitary, spherical, surrounded by a thin inner wall and by a remarkably thick exospore up to 15 μ thick, with a surface which is areolate and substellate from points and ridges. Antheridial branches of declinuous origin, slender, winding, sometimes branched. Antheridia small (19 $\times$ 15 μ) spherical or clavate, applied at the base of the oögonia and penetrating the oögonia by developing a fertilization tube.

On submerged fruits in lakes, ponds and ditches in stagnant water, March to December. Ann Arbor, Michigan. On surface of decaying fruits, Hamburg and Breslau, Germany (von Minden); in France (Cornu); in Denmark (Petersen).

(a) A form which is figured by von Minden (26, Pl. 2, fig. 10, and text figs. 9–10) was also observed by me at Ann Arbor. The plant consists of a thalloid placque, usually disk-shaped, appressed to the substratum, attached by rhizoids, provided on its periphery with very short sporangia-bearing stunted branches. Zoösporangia typical, usually smaller. Sexual reproductive organs never observed.

(b) Forma *attenuata* f. nov. (Plate XLVIII).

Vegetative plant very slender, attached by long, slender, penetrating rhizoids. Basal portion very long and narrowly subcylindrical, simple or sometimes once forked, 2000–3200 $\times$ 20–50 μ , wall relatively thick, 7–10 μ , protoplasm hyaline, including numerous oil globules upward. Branches scanty, arising from the apex of the main portion, differentiated at point of origin by pedicel-like constrictions which are provided with perforated cellulose deposits, very slender and flexible, 200–500 $\times$ 6–9 μ .

Asexual and sexual reproduction typical.

On decaying Crataegus and apple fruits submerged in stagnant water. Ann Arbor, Michigan, October and November, 1925.

Unique by its appearance on the substratum where it forms a grayish-white, interwoven, thick, dense mat, while all other specimens are tufted or solitary in habit.

RHIPIDIUM AMERICANUM Thaxter, Bot. Gaz. 21: 320. Pl. 22,
figs. 1-15. 1896

Kryptogamenfl. Mark Brandenburg 5: 599; Mycol. Untersuch. 2: 188.
Pl. 3, fig. 21.

Vegetative plant attached to substratum by means of copious penetrating rhizoids. Basal portion variable in form and size. 75-400 μ above, more or less regularly one or more times successively dichotomously branched or lobed, the lobes or branches erect or spreading in a radiate fashion; branches numerous, 50-800 $\times$ 12-14 μ , arising from the upper or external edges of the basal portion, sometimes consisting of two or three subclavate segments, arising from the basal cell by pedicel-like constrictions.

Asexual reproduction by means of zoösporangia originally terminal, one to four succeeding one another on a single filament, rarely two or three borne terminally, varying in form, typically ovoid, 30-90 $\times$ 20-46 μ (30-86 $\times$ 20-27 μ , Thaxter), apex bluntly rounded. Zoöspores biciliate, composed of coarse refractive granular protoplasm, emerging from the sporangium in a cylindrical mass surrounded by a thin membrane and surmounted by a papilla of dehiscence, monoplanetic, 10-12 μ .

Sexual reproduction by means of oögonia and antheridia; oögonia borne terminally on branches with suboögonial pedicel-like constrictions like those of the sporangia, spherical, 40-55 μ in diameter; oöspore solitary, colorless, thick-walled, 30-45 μ in diameter, with exospore elevated in a series of anastomosing ridges which give the spore an irregularly stellate outline; antheridial branches arising from the same branch that bears the oögonium, and immediately beneath it, short, slender; antheridium small, 14 μ , rounded, applied close to the base of the oögonium. Fertilization tube penetrating the oögonium.

On decaying submerged fruits of Crataegus, rose and apple. Ann Arbor, Michigan. March to December. On various decaying vegetable substances, Massachusetts, and Maine (Thaxter); Hamburg (von Minden).

This fungus appears to be a common plant in the vicinity of Ann Arbor. It has been obtained often growing on the same fruits with other rhipidiids and with blastocladias. It usually forms a dense tuft on the fruit surfaces, but sometimes grows singly.

RHIPIDIUM THAXTERI von Minden, Kryptogamenfl. Mark Brandenburg
5: 600. 1912

Falck's Mycol. Untersuch. 2: 188. Pl. 3, figs. 22-24.

Vegetative plant attached to substratum by means of numerous branching penetrating rhizoids. Basal portion often of large size, sometimes 800 μ broad with lobes up to 200 μ wide, or at times more cylindrical, quite thick-walled. Branches arising from the basal cell, of uniform thickness, about 7-11 μ , floating, somewhat flexuous, nearly always without intercalary segments, but constricted at point of origin and below the reproductive organs.

Asexual reproduction by means of zoösporangia, one or several formed terminally on a branch and usually also on very short lateral and distant supports which are acquired by a sympodial arrangement due to continued growth of the branch from a region just below the mature sporangium.

Sporangia long-ellipsoid, $50-55 \times 20-35 \mu$, opening at the apex; zoöspores escaping like those in *R. europaeum* and of similar form and structure.

Sexual reproduction by means of oögonia and antheridia. Oögonia borne on short somewhat spirally twisted stalks arising from the basal portion and gradually widening upward without suboögonial constriction, large, globose but mostly somewhat pyriform, $57-62 \times 45-57 \mu$, wall slightly rough-wrinkled at maturity. Oöspore solitary, large, thick-walled with stellar ridged exospore. Antheridial branches arising from the basal stalk, of declinuous origin, long-winding, branched, growing to the apex of the oögonium. Antheridial filaments slender, irregularly branched, arising mostly from the basal portion of the thallus among the other branches. Antheridia unusually large, $50-70 \times 7-11 \mu$, irregularly clavate, applied by their whole length to the oögonium, coalescing with the oögonial wall at its apex where it develops a penetrating fertilization tube.

On decaying fruits, Hamburg, Germany (von Minden).

Adapted from von Minden.

RHIPIDIUM PARTHENOSPORUM SP. NOV.

Vegetative plant attached to the substratum by means of a few rhizoids. *Basal portion* elongated and slender, once or twice forked, $800-1000 \mu$ long by $25-30 \mu$ wide; wall smooth, hyaline, not becoming greatly thickened, $7-10 \mu$ thick; protoplasmic contents hyaline, coarsely granular and containing many oil globules. *Branches* very short, arranged in umbels, constricted pedicel-like at point of origin, enlarged in an ellipsoid or globose manner above this constricted portion which bears the reproductive organs, constrictions with plug-like cellulose deposits.

Asexual reproduction by means of sporangia which are borne in umbellate clusters on the short branches, ellipsoid, thin-walled, $50-60 \times 34-50 \mu$, clusters few to several. Zoöspore formation unknown.

Sexual reproduction by means of oögonia in which are formed solitary, parthenogenetic oöspores borne in umbellate clusters on the short branches, with suboögonial pedicel-like constrictions, spherical, thin-walled, smooth, $52-54 \mu$ in diameter; during development differentiated into oöplasm and periplasm, the latter with strongly marked radiating periplasmic strands so as to appear cellular; mature oögonia not seen. Antheridia apparently lacking.

On decaying apple, immersed in the shore water of a lake. April. Type collected by Bessie B. Kanouse near Ann Arbor, Michigan.

There is little doubt but that this plant is a definite species although unfortunately it has not been found in sufficient quantity to determine all its characters, nor has its artificial culture hitherto been a success.

It is sharply marked by the manner in which it bears the sporangial and oögonial clusters on the same plant, but these organs were never mixed in any of the clusters in the plants seen. Stunted plants of related species may show similar reduction of the branches, but in such cases the basal portion is equally reduced, while here the short branches bearing the reproductive organs occur regularly on what must be considered as normal plants.

GENUS: ARAIOSPORA¹ Thaxter, Bot. Gaz. 2: 326. 1896

Mycelium forming a large cylindrical pseudo-cell bearing rhizoids and branches. Branches segmented, arising in whorls from the distal end, repeatedly umbellately branched, pseudo-cells cylindrical or nearly so. Zoösporangia of two kinds, the one smooth, the other differently shaped and furnished with prominent spines. Zoöspores emerging in a mass at first surrounded by a thin membrane which ruptures almost immediately, biciliate, monoplanetic. Oögonia borne in whorls or umbels, often associated with the zoösporangia, spherical, separated from the segments like the zoösporangia by a constriction. Oöspore solitary, exospore surrounded at maturity by a row of cell-like compartments of periplasmic origin. Antheridial branches arising from special segments, simple or branched. Antheridia small, rounded, applied close to the base of the oögonium. Fertilization tube formed by the oögonium from within.

ARAIOSPORA SPINOSA (Cornu) Thaxter, Bot. Gaz. 21: 590. 1896

Rhipidium spinosum Cornu, Ann. Sci. Nat. Bot. V, 15: 14. Pl. 5, figs. 1-9.—Kryptogamenfl. Mark Brandenburg 5: 593, figs. 10a-c.—Mycol. Untersuch. 2: 157. Pl. 1, figs. 1-8.

Vegetative plant attached to substratum by numerous penetrating rhizoids. Basal portion variable, often large, broadly cylindrical, $800 \times 160 \mu$, with stout walls, or in feeble plants thinner; at its upper end provided with numerous stout cell-like branches usually repeatedly umbellately branched, on which the branching may be repeated; constrictions only at the origin of the branches and at point just below the reproductive organs, seldom elsewhere. Primary branches $780 \times 76-96 \mu$.

Asexual reproduction by means of zoösporangia of two kinds. These are borne in whorls with two to six in a whorl; the smooth-walled sporangia more or less long-ellipsoid, $90-150 \times 45-60 \mu$; the spined sporangia oval, $100-150 \times 40-80 \mu$, or broadly ellipsoid, with mostly solid pointed spines which are straight or slightly bent, either stiff or weak, usually in two rows in an indefinite number, often arranged about the exit-papilla, size variable, $60-70 \times 9 \mu$. Zoöspores in both sporangia of the same form, kidney-shaped with two cilia attached laterally, monoplanetic.

Sexual reproduction by means of oögonia and antheridia. Oögonia formed in a clustered fashion on a constricted pedicel, spherical, wall smooth, brown. Oöspore solitary, not completely filling the oögonium, spherical, surrounded with a thick envelope of cellular structure. Antheridia borne on long, thin branches of diclinous origin, relatively large, curved-cylindrical, often encircling the oögonial wall.

On decaying immersed twigs of oak and of alder, upon decaying rhizoids of water lily, Breslau and Hamburg, Germany (von Minden); probably also in France (Cornu).

Adapted from von Minden.

¹See also Lindner, David H. A new species of Araiopora from British Guinea. Mycologia 18: 172-178. 1926.

ARAIOSPORA PULCHRA Thaxter, Bot. Gaz. 21: 328. Pl. 23,
figs. 20-25. 1896

Proc. Boston Soc. Nat. Hist. 31: 211-245.—Kryptogamenfl. Mark
Brandenburg 5: 598.

Vegetative plant attached to substratum by means of rhizoids. Basal portion variously developed, usually large, $1000-1500 \times 25-50 \mu$, subcylindrical, the ramiferous extremity subconical, bearing numerous acropneurogenous branches in a more or less distinctly umbellate fashion and separated from it by the usual constrictions. Branches composed of more or less cylindrical segments and repeatedly umbellately branched, the segments subcylindrical becoming more slender and usually longer as they succeed one another, branches long, $275-2750 \mu$.

Asexual reproduction by means of zoösporangia of two types. Zoösporangia borne in whorls or umbels; one type subcylindrical or broadly clavate, smooth, $120-275 \times 30-35 \mu$; the second type broadly oval to pyriform and furnished with large spines radiating in all directions, but sometimes short and stout and confined to the distal extremity. Spinose forms $48-70 \times 45-60 \mu$. Spines $10-35 \mu$ long. Zoöspores escaping through a wide exit-papilla, finely granular, biciliate, monoplanetic, emerging in a mass at first surrounded by a thin membrane which ruptures almost immediately.

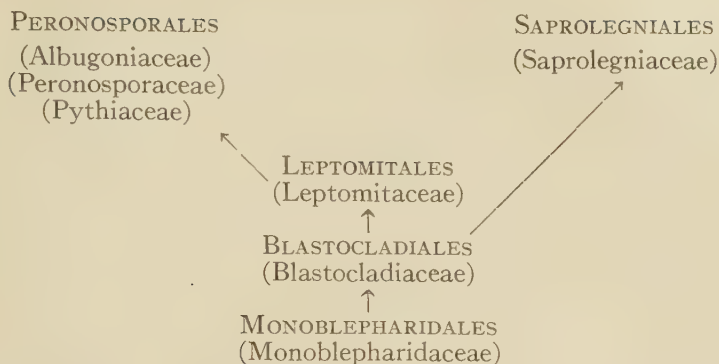
Sexual reproduction by means of oögonia and antheridia. Oögonia borne like the sporangia, the constricted pedicels which separate them from the segments very short, $50-60 \mu$. Oöspores spherical, $35-45 \mu$ thick, wall colorless, surrounded by a single layer of more or less hexagonal, peripheral cells derived from the periplasm, cells about $7-10 \mu$ in diameter. Antheridial branches arising from special segments, simple or branched, the small rounded antheridia applying themselves close to the base of the oögonium. Fertilization tube initiated and formed by the oögonium.

On sticks, Massachusetts (Thaxter).

Adapted from Thaxter (l. c.) and from King (18).

PHYLOGENETIC CONSIDERATIONS

All of the systematic arrangements that have been suggested for this group of fungi fall short of presenting an adequate picture of the phylogeny of the water molds. The following arrangement is proposed in the belief that it indicates the natural affinities existing between the several orders, and it is founded upon the morphological facts as we know them at present. The interpretation involves the erection of a new order, the LEPTOMITALES. The following diagram presents the scheme of this new phylogenetic arrangement. It is succeeded by a discussion of the relation of the orders and of their integral parts, together with a presentation of the significant facts that lend support to this proposal.



As is shown in the diagram, the relationship of the Leptomitales at the lower limit is with the Monoblepharidales through the Blastocladiates, while at the upper limit it is with the Peronosporales.

The most recent and by far the most comprehensive criticism of the older taxonomic views is given by Butler (4). He summarizes the several important phylogenetic schemes that have been proposed for the classification of the Oömycetes since the time of de Bary (11), and makes clear that not only de Bary, but Brefeld and Fischer were mistaken in placing *Pythium* as the starting point in the phylogenetic line of this group of fungi. Butler's discussion is centered primarily around the genus *Pythium* with a strong defense for the belief that this genus represents an advanced rather than a primitive position in relation to the other Oömycetes. He discusses views held by the previous investigators concerning the position of the family Leptomitaceae and concludes by saying "we are justified in considering that a derivation of the main families through Monoblepharis and the Leptomitaceae has most in its favor."

This view was reëmphasized and strengthened by Butler (5) in a later paper on Allomyces, a genus included by him in the Leptomitaceae.

Thus Butler has paved the way in a logical and clear manner for the further reorganization of the Leptomitaceae and Blastocladiaceae into the arrangement here proposed. He has not, however, suggested any classification that would correct some of the difficulties associated with the two families mentioned above.

These families have usually been included in the order Saprolegniales. Thaxter is the only botanist who has intimated any other possible relationship for the Leptomitaceae. He included the family in that order but with the following qualification: "Should the family be united with any other, it must be with the Pythiaceae, if we recognize them as distinct from the Peronosporaceae, or with the latter if we do not." Since he did not make clear his conception of the position of the Pythiaceae, his conclusion remains uncertain. Only indirectly does he suggest a complete removal of the Leptomitaceae from the Saprolegniales. If, as was suggested, that family were united with the Pythiaceae, this would not necessarily mean its

exclusion from the Saprolegniales, as the Pythiaceae were included in the Saprolegniales by Schröter. On the other hand, if the Pythiaceae were considered a family of the Peronosporales, as was proposed by de Bary (11), it would mean a complete removal from the Saprolegniales.

A brief summary of the characters of the orders already established is necessary before the similarities and differences between the old orders and the proposed new order Leptomitales can be fully recognized.

The Peronosporales comprise a group of fungi that are commonly parasitic; they may reproduce asexually by conidia; sexual reproduction is by means of oögonia, and antheridia with fertilization tubes. The oögonia contain single oöspores surrounded by periplasm.

The Saprolegniales are a group of water-inhabiting fungi commonly saprophytic. They reproduce asexually by means of zoöspores and by gemmae. Sexual reproduction is by means of oögonia in which many oöspores without periplasm are formed, and antheridia with fertilization tubes.

We shall now consider critically the asexual processes in the three orders and show, first, why the Leptomitales, while allied to the Saprolegniales through the formation of similar types of zoösporangia, can not be included with them.

Throughout the Saprolegniales typical zoösporangia are formed commonly and abundantly. The zoöspores are biciliate and diplanetic. In the Leptomitales there are also abundant zoösporangia and the zoöspores are likewise biciliate. However, a further comparison will show that the behavior of the zoöspores is not entirely alike in the two orders. The zoöspores in the Leptomitales are monoplanetic instead of diplanetic. As far as known this is true of all of the genera excepting Leptomitaceae and Apodachlya. Hence one of the arguments for including the family Leptomitaceae in the Saprolegniales on the basis of analogous zoösporangia does not hold good in its entirety. The condition of diplanetism is usually considered to represent a higher degree of specialization than is expressed by monoplanetism. Whether this be true or not does not alter the fact that the asexual reproductive processes in the two orders, though somewhat comparable, exhibit fundamental differences. If the question of including the Leptomitaceae in the Saprolegniales depended entirely on this one matter of zoöspore behavior, it probably would lose some of its significance, but when to this is added the segmentation of the hyphae and the presence of oögonia containing single oöspores with periplasm, we have important reasons for believing that the family Leptomitaceae should be placed in a distinct order and that it does not have a close relationship with the Saprolegniaceae.

In the asexual processes in the Peronosporales and Leptomitales we find even greater differences than those mentioned above. In the Peronosporales asexual reproduction may take place by means of conidia which germinate

through formation of one or more germ tubes. In the genus *Phytophthora* and elsewhere the conidia are capable under certain conditions of functioning as zoösporangia. To be sure, these conditions may be commonly present but this does not invalidate the point just made. In the genus *Pythium* there is abundant evidence, as Butler (4) has pointed out, for believing that the zoösporangia and conidia are homologous. The same view doubtless applies to other genera in the Peronosporales. In some of the land-inhabiting pythiums conidia may be formed almost exclusively, while on other water-inhabiting species only zoösporangia are produced. Further proof of the homology of these two organs is given by the fact that a mere change in external conditions can determine whether the organ shall function as a conidium or as a zoösporangium (4). However, in the genus *Peronospora* the potentiality for developing in either direction has been practically lost. Hence one can say, physiologically speaking, that the usual means of asexual reproduction in the whole order is by conidia. In the Leptomitales asexual reproduction is exclusively by means of zoöspores formed in typical sporangia. These organs have never been known to function as conidia. This is possibly due, in part, to the fact that all of the fungi included in this order are water-inhabiting and hence have never developed the conidial condition so often associated with fungi parasitic on land plants. Until we are able to study these forms intensively in culture, we can not know whether or not the conidial condition may be induced under certain circumstances. Since such types of asexual conidia are not known to occur anywhere in the Leptomitales, it would be illogical to combine the two orders Peronosporales and Leptomitales. The relationships that do exist are based on other morphological grounds.

Asexual reproduction in certain of the Oömycetes may take place by the production of gemmae or chlamydospores as well as by zoösporangia or conidia. Butler defines a gemma in *Pythium* as a "vegetative bud representing a portion of the mycelium containing within itself a sufficient store of reserve nutrient to enable it to withstand isolation from the plant without losing its power of renewed growth." He followed Maurizio (24) in considering the gemmae, as just described, to be peculiar to the genus *Pythium* and not comparable to the "gemmae" known in the Saprolegniales. The presence of gemmae in the Leptomitales, however, is unknown. What are loosely called "resting spores" are not equivalent to the gemmae (see morphological discussion in the present paper).

Turning now to a consideration of the sexual processes in the several orders it is clear that there are good evidences for claiming relationships between the Peronosporales and Leptomitales. In the Peronosporales and Saprolegniales the process is markedly different. In the Saprolegniales many oöspores without periplasm are commonly formed in each oögonium. This fact at once precludes any possibility of including in that order any fungi in which single oöspores with periplasm are formed—a condition found throughout the Leptomitales.

In the sexual reproductive processes in both the Leptomitales and the Peronosporales only the one oöspore with periplasm is formed. This type of development was observed and described in the Peronosporales by de Bary (10, 11), Büsgen (3) and others. They recognized the presence of a specialized protoplasm in the central part of the oösphere which was surrounded by a layer of thinner protoplasm. The problem of the detailed structure and function of the oöplasm has been a favorite topic for cytological investigation. Some of the strongest evidence that there are homologies between the two orders has been derived from cytological studies on the genera *Pythium*, *Phytophthora*, *Peronospora* and *Albugo* in the Peronosporales, and on *Araiospora* in the Leptomitales.

A brief summary of the facts, so obtained, that are essential to this discussion center around the following points: (1) presence or absence of a fertilization tube; (2) methods of formation of the fertilization tube; (3) presence of a receptive papilla on the oögonial wall; (4) formation of a central body in the oösphere; (5) development of the protoplasm into oöplasm and periplasm.

Concrete instances will now be examined which have resulted from cytological and morphological studies and which show why there is strong evidence for believing that the Leptomitales and Peronosporales are related, even though (for the other reasons previously discussed) they can not be united.

Early morphological investigations had shown that in the Peronosporales definite fertilization tubes of antheridial origin are formed (8, 11). Later cytological studies confirm this and actual fertilization also has been established. For this data we depend upon the substantial cytological contributions on *Peronospora* by Wager (37) and Whetzel (38), and on *Albugo* by Stevens (29) and Davis (9). The presence of fertilization tubes of just such undoubted antheridial origin are also clearly present in *Rhipidium* and *Sapromyces* in the Leptomitales. In *Rhipidium europaeum* and in *R. americanum* the fertilization tubes are plainly seen inside of the oögonia extending into the dense protoplasm at the center. After the periplasm has contracted to form the exospore, the tube stands out distinctly in sharp relief. Thaxter describes and figures (33) a similar condition for *Sapromyces androgynous*, and von Minden (25) gives ample proof of fertilization tubes of this nature in *S. Reinschii*.

King (18) working on *Araiospora pulchra* failed to find a fertilization tube of the type just mentioned, but describes and figures a definite tube which is formed entirely by the oögonium. While the formation of a tube in exactly this manner is quite unlike anything known in any other Phycomycetes, King remarked that in *Albugo* a similar modification is found in which the oögonium seems to take some part in the formation of the fertilization tube (36).

In all the cases known in the Leptomitales, the antheridial tubes enter

at definite places on the oögonial wall, but not necessarily at any specialized area on the wall. In *Sapromyces*, Thaxter (34) states that the wall at the apex of the oögonium becomes indented by the antheridium. This is the nearest approach to a receptive papilla such as has been reported for several species of *Albugo* by Stevens (29) and Davis (9). However, a receptive papilla, or even a definite area on the oögonial wall through which the fertilization tubes enter, is generally lacking in the *Saprolegniaceae*. Butler (4, p. 52) claims that receptive papillae are present in *Aphanomyces* spp.

Still another morphological character common to the two orders *Peronosporales* and *Leptomitales* is the formation in certain species of a differentiation in the oöplasm itself. Such a specialized part found in species of *Albugo* and called the *central body* by Wager (36), and the *coenocentrum* by Stevens (29), is supposed to consist of trophoplasm which functions in uniting the two gametes. A similar structure was again described in great detail for *Araiospora pulchra* by King (18).

The last and most important of all of the evidence submitted concerns the presence of periplasm in the oögonium during and after oögenesis. It is unnecessary to describe here its appearance in the oögonia, as that has already been done adequately by many investigators. It is sufficient to state that, as far as it is known, the oögonia in all or nearly all of the genera in the *Leptomitaceae* (33), *Pythiaceae* (4), *Peronosporaceae* (36), and *Albugoniaceae* (29), produce such a differentiation of protoplasm during the formation of the oöspore. It is in all probability much alike in its nature and function in all of these families. After fertilization the periplasm seems to disappear or to be altered and sometimes it forms what is commonly called the roughening of the exospore. In certain genera it forms radiating strands between spore and oögonial wall and becomes so characteristic a feature that it can be used as a good generic character. This has been done in describing the genera *Rhipidium* and *Araiospora*. In the former the periplasm later contracts and forms a ridged stellar layer on the exospore while in *Araiospora* the strands persist and form a single row of cell-like chambers surrounding the oöspore.

Still another characteristic by which the *Leptomitales* are distinguished from the *Saprolegniales* and *Peronosporales* is by the presence of constrictions in the hyphae. Associated with the constrictions are the pseudo-septa which were early a source of interest to botanists. The pseudo-septa are composed of "cellulin," a substance so named by Pringsheim. It gives a negative reaction with the usual tests for fungus cellulose. The presence of these pseudo-septa was later made one of the chief characterizations of the *Leptomitaceae*. Thaxter describes these peculiarities of the family as a "segmentation of the hyphae through the presence of successive constrictions each corresponding to a pseudo-septum formed by a deposit of 'cellulin' (?) which nearly closes the passage from one segment to another except for a central perforation through which the protoplasm of the adjacent segment

may usually be seen to be continuous." That distinction is retained as a character of the order Leptomitales. Although it is only a vegetative character, it is good additional evidence for keeping the Leptomitales apart from the other orders.

The facts here set forth show clearly that the Peronosporales and Leptomitales are allied in the character of their sexual reproductive organs, and that the Saprolegniales and Leptomitales are not closely allied in these traits. The segmentation of the hyphae also precludes a combining of the Leptomitales with either of the other two orders mentioned. The relationships throughout the whole group of Oömycetes are much more easily understood by accepting the order Leptomitales as a distinct order intermediate between the Blastocladias and the Peronosporales.

The systematic relations within the family Leptomitaceae have been fully discussed by Thaxter (31, 32, 33, 34) and von Minden (25, 26) and need not be considered again here.

Another change affecting a portion of this group of water molds was made in 1910 by Petersen (27). At that time he established the order Blastocladias. In it he placed his new family Blastocladiaceae with one genus, Blastocladia. That arrangement appears for the first time thereafter in von Minden's classification in 1916. The latter retained Petersen's arrangement and added Allomyces to that family. Such a classification does not meet all of the taxonomic difficulties in a satisfactory way, for it still leaves untouched some of the vexing problems pertaining to the relationships of the Leptomitaceae, Blastocladiaceae and Saprolegniaceae that have existed for a long time.

The Blastocladiaceae have, in common with the Saprolegniaceae, certain characteristics that seem to have been obscured by previous arrangements of the Phycomycetes. Such are the absence of periplasm in the formation of oöspores; the production of large numbers of zoöspores, and the absence of constrictions in the hyphae. It must be conceded that there are still gaps in the present arrangement of the groups so far discussed but these may become bridged more and more by the discovery of new forms. The fungi known at present do not provide an unbroken series, but nevertheless the evidence at hand seems to support the phylogenetic scheme just indicated.

The order Blastocladias as here constituted follows the conception established for it by Petersen (l. c.). Within this family are found the fungi with characteristics making the transition from the Monoblepharidales reasonably clear. The chief characteristic of the order Monoblepharidales, namely the presence of motile antherozoids, is so unique that from the present data neither the Leptomitales nor the Blastocladias could be combined with it.

This classification is based largely upon vegetative and asexual reproductive processes and is subject to modification whenever sufficient evidence

warrants a change. Our lack of data concerning the sexual reproductive processes makes necessary the use of other facts in formulating a systematic scheme. Nevertheless, that basis of classification is recognized as having phylogenetic significance in such plant groups as the algae, and should be rated accordingly here.

The organization of the Blastocladiaceae differs in several respects from any previous arrangement. In addition to Blastocladia and Allomyces, the genus *Gonapodya* has been included and a new genus, *Mindeniella*, has been added.

The family so constituted reemphasizes the close relationship between that family and the Monoblepharidaceae. Butler (5) gave an excellent discussion of the relations between the three genera *Gonapodya*, *Blastocladia* and *Allomyces* and their relation to *Monoblepharis*, without considering Petersen's new order, which had appeared only a short time previously. He placed the first three genera in the Leptomitaceae, but regarded them as a special group in that family.

The genus *Gonapodya* is included chiefly because of its non-cellulose walls, and because its zoöspores possess a fluctuating number of cilia, frequently one.

The genus *Mindeniella* exhibits most of the characters just mentioned, but differs in having membranes of cellulose composition and in having the sporangia and oögonia supported on pedicels. Its strongest alliances with the family lie in the general habit of growth, the lack of definite constrictions, the lack of periplasm and the large zoösporangia producing large numbers of zoöspores. It is probably one of the connecting links between the Blastocladiales and the Leptomitales.

*Pythiomorpha*² clearly differs from the Leptomitales by the lack of constricted hyphae and cellulose plugs; and from the Blastocladiales by its biciliate, diplanetetic zoöspores and filamentous thallus. It is evidently more closely related to *Pythium* by its biciliate zoöspores, filamentous thallus and similar mode of sexual reproduction, but differs decidedly in the structure of the mature sporangium, which lacks a tube or specialized beak such as occurs in the sporangia of all of the species of *Pythium*. Moreover the zoöspores do not form in an exterior vesicle as in that genus. They are also truly diplanetetic, whereas in most of the species of *Pythium* the zoöspores are monoplanetic or have a specialized diplanetism (4).

Stevens (30), influenced by Fitzpaterick's view (12), has extended the limits of the Pythiaceae to include the three genera *Pythium*, *Pythiocystis* and *Phytophthora*. With this modification of Schröter's conception of the Pythiaceae, the family takes on a much more important place in the classification, and stands out in clearer contrast with the Albuginaceae and *Pero-*

² A paper by Christine Johanna Buisman (Root rots caused by Phycomycetes. Haarlem, F. P. Visser, 1927) appeared after this paper went to press. It is plain that Miss Buisman has not given sufficient attention to the characters of the genus *Pythiomorpha* or she would not suggest placing that genus with *Phytophthora* on the slight evidence which she presents.

nosporaceae. Stevens' arrangement is unsupported by any accompanying remarks or discussion, but appears to be a step toward a more logical and lucid system within the Peronosporales. The purely physiological facts known for the species of the genera *Phytophthora* and *Pythium* set them off from the downy mildews as such. The saprophytic life, for example, which the species of these two genera can lead, in itself binds them together in contrast to the more fixed parasitism of the genuine downy mildews.

With this newly extended conception of the limits of the Pythiaceae, it at once becomes clear that *Pythiomorpha gonapodioides* has many things in common.

In a previous paper (16) it was shown that under the then existing taxonomic arrangements, *Pythiomorpha* properly belongs in the family Pythiomorphaceae erected for it by Petersen (27). If we accept the introduction of *Phytophthora* into the Pythiaceae and the widening of the gate to that family in order to permit its entrance, than it is equally possible to lead the genus *Pythiomorpha* into the same fold, and Petersen's family becomes superfluous. Without deciding the status of *Pythiocystis* any further than was done by Stevens, the Pythiaceae are here considered to include *Pythium*, *Pythiocystis*, *Phytophthora* and *Pythiomorpha* as well as von Minden's *Pythiogeton*.

The relationship of *Pythiomorpha gonapodioides* seems closer to *Phytophthora* than to the genus *Pythium*. As already indicated it differs in three important particulars from *Pythium*; from *Phytophthora* it differs in its diplanetic zoöspores, in the absence of any conidial stage, and in its proliferating sporangia. In other characters it shows clearly its affinity with the Peronosporales as a group. The sexual organs, the mode of fertilization, and the presence of oöplasm and periplasm in the developing oögonium are equivalent to those of that order. On the other hand, there is little doubt that it shows considerable relationship to the Leptomitales, and it perhaps represents a connecting link with that group.

SUMMARY

1. By a study of more than half of the species of the Blastocladiaceae, Leptomitaceae, and Pythiomorphaceae now known, a rearrangement of the fungi belonging in these groups has become necessary and a new phylogenetic classification is here presented.

2. A new order, the Leptomitales, is shown to maintain a phylogenetic position between the Blastocladales on the one side and the Peronosporales and Saprolegniales on the other side.

3. A new genus, *Mindeniella*, has been discovered, studied and described, and its position in the Blastocladiaceae determined.

4. Five new species have been found and described: *Mindeniella spinospora*, *Blastocladia gracilis*, *Blastocladia tenuis*, *Blastocladia globosa*, and *Rhipidium parthenosporum*.

5. In *Pythiomorpha gonapodioides*, which was a part of this series of studies and an account of which has already been published (15), it has been shown that no vesicle is present at the time of the escape of the zoöspores. The sexual mode of reproduction in this species is for the first time definitely established.

6. *Pythiomorpha* is here shown to belong definitely to the Pythiaceae as emended by Stevens (30).

7. It is demonstrated that at least in one species of the Blastocladales antheridia are formed, although hitherto male organs in this group had not been known to exist.

8. A study of the structure of the so-called "resting spores" in the genus *Blastocladia*, and the finding of antheridia in one species, has convinced the writer that such bodies are parthenogenetically formed.

9. The physiological experiments on the species belonging to the two genera *Rhipidium* and *Blastocladia* settle definitely the fact that these fungi require a special technique for their isolation into pure culture and that their reactions to environmental influences during development make necessary a decidedly different technique than that hitherto employed in obtaining pure cultures of the members of the Saprolegniaceae.

10. An improved method is described for collecting some of these aquatic fungi.

11. It is demonstrated that these forms are everywhere, and for the most part abundantly, present in our lakes, rivers, ditches, and ponds.

12. It is shown that these fungi can be kept and developed in rough cultures for long periods and that the plants that thrive under such conditions are, as far as can be determined, developed in a normal and healthy manner.

13. With all the new facts at command, it has seemed desirable to present these groups in an up-to-date monographic form, and to provide artificial keys.

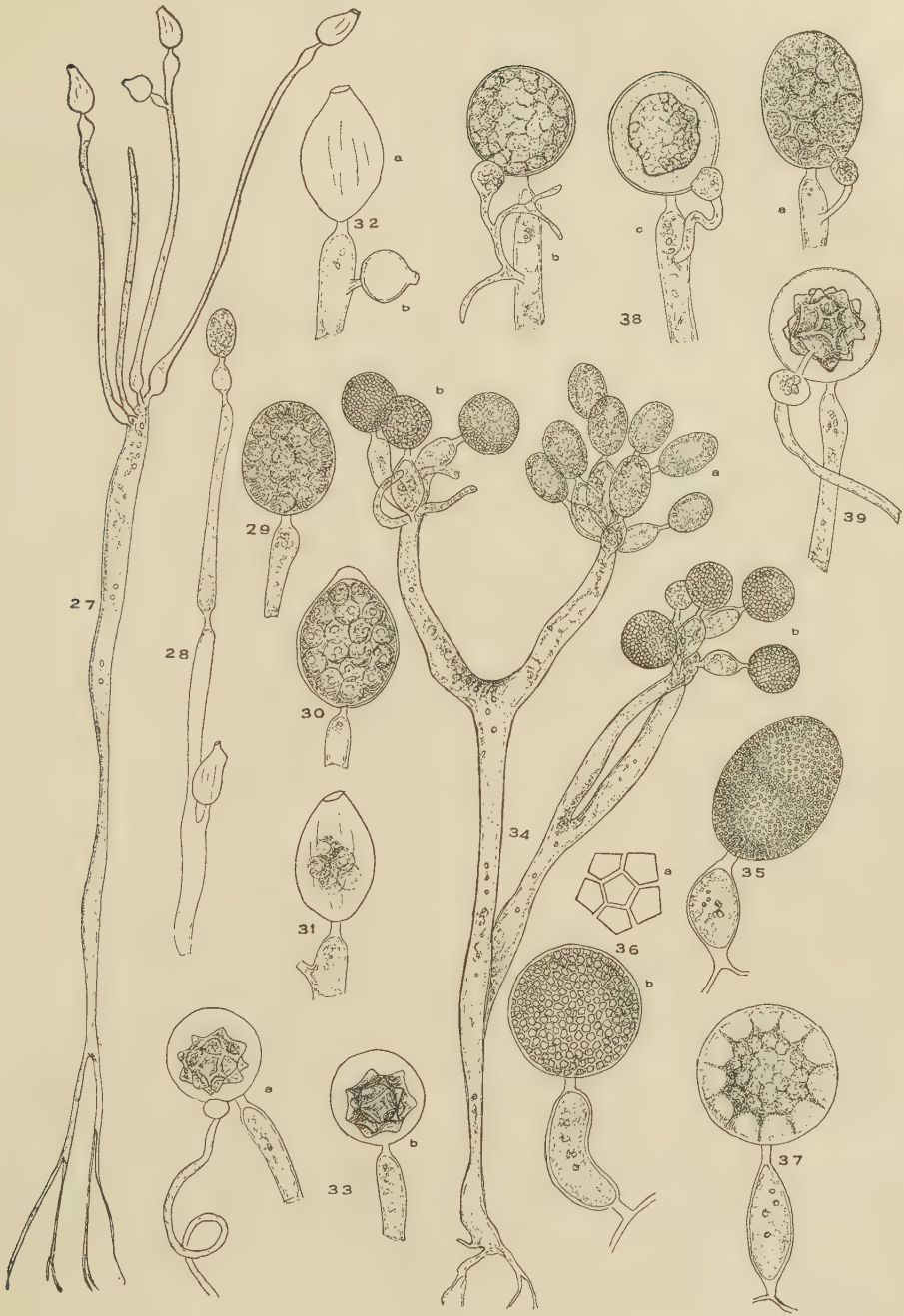
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DESCRIPTION OF PLATE

PLATE XLVIII

Figures 27-33, *Rhipidium europaeum* forma *attenuata*. Figures 34-37, *R. parthenosporum* sp. nov. Figure 38, *a*, *b* and *c*, *R. americanum*. Figure 39, *R. europaeum*.

FIG. 27. A whole plant.

FIG. 28. Branch taken from upper portion of plant shown in figure 27.

FIG. 29. Zoösporangium giving the appearance of the protoplasm before the spores are formed.

FIG. 30. Zoösporangium in which the spores are almost ready to be released. The papilla of dehiscence is plainly visible.

FIG. 31. Sporangium from which a few spores failed to make their escape.

FIG. 32. A portion of a filament bearing two empty zoösporangia; *a*, a thin-walled sporangium; *b*, a thick-walled sporangium.

FIG. 33. *a*, an oögonium containing a mature oöspore. An antheridium of diclinous origin is seen attached to the oögonium. *b*, oögonium containing a mature oöspore.

FIG. 34. An entire plant terminated by whorls of short branches bearing reproductive organs. *a*, zoösporangia; *b*, oögonia.

FIG. 35. A zoösporangium in which the protoplasm is still in a very finely divided and evenly distributed condition.

FIG. 36. *a*, a detail of the arrangement of the protoplasmic contents in an early stage of the development of a sporangium; *b*, a stage in the development of a parthenospore.

FIG. 37. A later stage in the development in which the conspicuous protoplasmic strands radiating from the dense central mass to the oögonium wall are plainly visible.

FIG. 38. *a*, *b* and *c*, oögonia showing different stages in the development of the oöspore. Note the antheridia of androgynous origin. *a*, an early stage in the development of the oöspore in which the protoplasm is distributed evenly throughout the oögonium; *b*, a later stage in which a differentiation of the protoplasm is becoming visible; *c*, a still later stage in which the oöplasm and periplasm are distinct.

FIG. 39. An oögonium and antheridium. The oöspore is fully developed and is surrounded by the characteristic wall giving the spore an areolate appearance. The fertilization tube is evident. The antheridial branch is of diclinous origin.

